بسم الله الرحمن الرحيم

UNIVERSITY OF KHARTOUM

THE GRADUATE COLLEGE

MEDICAL & HEALTH STUDIES BOARD

Relation of Body Fat Distribution and Lipid Profile to Plasma Level of Inflammatory Markers

By

HANADI ABD ELGADIR AHMED
B.Sc.

A thesis submitted in fulfillment of the requirements for MSc. degree in Human Physiology

Supervisor

DR. AMAL MAHMOUD SAEED M.B.B.S., Ph.D.
Associate Professor of Physiology
Department of Physiology
Faculty of Medicine
U of K

Jan. 2009

CONTENTS

CHAPTER ONE

Page

Page

CHAPTER TWO

Page

CHAPTER THREE

CHAPTER FOUR

Dedication

To soul of my father

Acknowledgement

In the Name of Allah, the Beneficent, the Merciful. Praise is to our God, Allah, the Lord of the world, who made me strong and gave me power to do this work.

It is my pleasure to express my sincere thanks and gratitude to Dr. Amal Mahmoud Saeed, Department of Physiology, Faculty of Medicine, University of Khartoum, for supervising this work and providing me with unique opportunity to work with her. I do appreciate her assistance, patience, continuous support, open minded comments and valuable suggestions during this study.

I wish to thanks Dr. Mouna Hamid, Medical Sciences Division, National Research Center, Egypt, for the kind help and excellent technical assistance.

I am very indebted to all the staff of the Department of Physiology, Faculty of Medicine for their co-operation, advices and support during the course of the study. Special thanks to Dr. Amir Abushouk, Dr. Nadia Omar, Dr. Tarig Hakeem, and Mr, Awad Gabir.

Deep gratitude are due to Prof. Tarig A. Abd El Rahman, Dr. Osama M. Osman, Dr. Salah O. Basheer and Dr. Atif Hassan Kher El Seed for their critical reading of the thesis and their great help and valuable suggestions.

I would also like to take this opportunity to express thanks to Mr. El Fatih Ballal, Mr. Hisham Hamed, Dr. Soher Shoummo, Ms. Magbola Mamoun, Dr. Mouna Faisal and Ms Shwida El Nour for their unlimited support.

I am very pleased to thanks Mr. Omar Osman and Dr. Ahmed AL Ramadi for their great help and endless co-operation.

l owe special thanks to my family. Much thanks to my mother, she has been thoroughly supportive for my goals. Thanks are extended to my sisters Hana and Shadia for helping me.

I owe my dearest thanks to my husband Adil, who has patiently shared all the pressure of this work and has given me great understanding and support. I am especially grateful to our children Mohammed, Maryam and Mayada, for filling my days with joy and happiness and for reminding me of what is important in life.

I would also like to express my gratitude and appreciation to everyone who has participated in this study.

Abstract

Background: Obesity is well known risk factor of metabolic complications and cardiovascular disease (CVD). On the other hand, C- reactive protein (CRP) and interleukin-6(IL-6) as markers of inflammation has lately aroused huge interest as predictors of CVD risk factors. Associations between anthropometric measurements of fat distribution and risk factors for CVD have been demonstrated in several populations.

Objectives: The aim of this study was to examine the association of various adiposity measures including total body adiposity [Body mass index (BMI) and percentage body fat (% BF)] and body fat distribution (waist, hip, wrist and thigh circumference.) with lipid profile and inflammatory markers (CRP and IL-6) in young females (age range 18-22 year).

Materials and methods: 224 female medical students were enrolled in the study after having their consent.

The anthropometric measurements including weight, height, waist, wrist, thigh and hip circumference and skin fold thickness were performed using standard procedures recommended by the international obesity task force (IOTF), WHO.

Body mass index (BMI) (kg/m^2), percentage body fat (% BF) and waist/hip ratio (WHR) were calculated. Subjects with BMI $\geq$30 kg/m2 were considered as obese (n= 64), while subjects with BMI $\leq$ 24.9 - $\geq$18.5 were considered as non- obese (n= 114).

Blood pressure was measured in all participants. Biochemical parameters, including total cholesterol, HDL-cholesterol, LDL- cholesterol,

triacylglycerols (TAG), fasting blood glucose and the inflammatory markers (CRP and IL-6) were estimated after 12 hours fasting.

Results: All anthropometric measurements except the height showed higher records among obese than in non-obese females (*P value* <0.05).

The mean ± SEM of BMI was found to be 20.6 ± 0.20 and 36.1± 0.6 for non-obese and obese female respectively. It was significantly higher in obese females (*P value* <0.01).

The study showed that the percentage of obesity among the participants 39.1% at BMI cut-off point >30kg/m^2. Classification of participants by the `% BF obtained by skinfold thickness from four points increased the percentage of obesity the participants to 51.5% at cut-off point >30%.

In 26.7% of the participants the waist circumference was >88 Cm and 94.6% of them their WHR of <0.85. Only 14.5% of the obese females with WHR of >0.85, Decreased WHR is attributed mainly to increased hip circumference (HC) of the study participants. Hip circumference in non-obese and obese females was found to be 100.73 ± 0.62and 124.77 ± 1.27 cm respectively.

Blood pressure was measured for all participants during systole and during diastole, and it was found to be high in 21.8 % of obese females.

Fasting blood glucose was found to be normal in all participants.
The study showed that there were higher values of lipid profile parameters in obese compared to the non-obese females. The mean ± SEM of total cholesterol, HDL, LDL, and triacylglycerol in obese females was 148.2 ±3.03, 37.4±1.4, 92.7± 3.33and 90.8 ± 4.41mg/dl respectively, while the mean ±SEM of these parameters in non-obese females was found to be

136.0 ± 3.23, 44.1±1.2, 76.9 ± 3.43and 75.51 ± 3.71mg/dl respectively. All these values were within the normal physiological ranges.

The study showed higher CRP in obese compared to the non-obese females, the mean ± SEM for non-obese and obese females was 0.305 ± 0.09and 6.256 ± 0.78 respectively this difference was statistically significant (*P value* <0.008). No difference in interleukin-6 (IL-6) levels was observed between them. The mean ± SEM of IL-6 for non-obese and obese females was found to be 5.76 ± 0.34and 5.16 ± 0.73 respectively (*P value* 0.101).

Conclusions: These findings indicated that fats in the obese females accumulated mainly in the lower part of the body rather than the abdominal region. Larger thigh and hip circumference could reflect increase femoral and gluteal subcutaneous fat; these depots have relatively high lipoprotein lipase activity and relatively low rate of basal and stimulated lipolysis. In addition, the majority of the studied obese females had normal blood pressure, normal fasting blood glucose and normal lipid profile; accordingly they are metabolically healthy but obese (MHO).

المستخلص

خلفية : تعتبر السمنة إحدى عوامل الاختطار للمضاعفات الاستقلابية والامراض القلبية – الوعائية. من جانب آخر فإن البروتين سي والإنترلوكين- 6 كمؤشرات إلتهابية قد أثارتا في الأونة الأخيرة إهتماماً كبيراً كمنبئات لإختطار القلبي الوعائي. وقد تم إيضاح الروابط بين القياسات البشرية وتوزيع الشحوم في الجسم والاختطار القلبي الوعائي في عدة مجموعات سكانية.

الاهداف: هدفت هذه الدراسة لاختبار الارتباط بين عدة قياسات للسمنة متضمنة السمنة الكلية للجسم (و مؤشر كتلة الجسم) و نسبة الدهون في الجسم وتوزيع الدهون في الجسم (محيط الخصر، محيط الحوض و محيط الفخذ) مع الوصمات الدهون و المؤشرات الالتهابية (بروتين سي التفاعلي و إنترليوكين- 6) للفتيات في المدى العمري ما بين 18 – 22 سنة.

المواد والأساليب : تم تضمين 224 فتاة من طالبات كلية الطب في هذه الدراسة بعد اخذ موافقتهم . أجريت القياسات البشرية شاملةً الوزن، الطول، محيط الخصر، محيط الفخذ، محيط الحوض و سمك ثنية الجلد باستخدام الإجراءات الدولية الموحدة التي أوصت بها منظمة الصحة العالمية و منظمة العمل الدولية للبدانة (IOTF) .

تم حساب مؤشر كتلة الجسم والنسبة المئوية للدهون في الجسم و نسبة محيط الخصر للحوض(WHR) تم اعتبار الأشخاص الذين احرزوا مؤشر كتلة الجسم$\leq$ 30 كجم/م^2 على أنهم يعانون من السمنة المفرطة (عددهن= 64) ، في حين أن الأشخاص مع مؤشر كتلة الجسم بين 24.9 - 18.5 إعتبروا طبيعيين (عددهن= 114).

وتم قياس ضغط الدم لجميع المشاركين.و تم قياس المعالم الكيميائية ، بما فيها الكوليسترول الكلي، كوليسترول - البروتين الدهني العالى الكثافه، كوليسترول – البروتين الدهني المتدنى الكثافه، الدهون ثلاثية الجلسريدات، مستوى السكر في الدم و المؤشرات الالتهابية (بروتين سي التفاعلي و إنترليوكين- 6) بعد 12 ساعة من الصيام .

النتائج : جميع القياسات البشرية، في ما عدا الطول، أظهرت سجلات أعلى فيما بين الإناث اللائي يعاننن من السمنة مقارنة بذوات الاوزان العادية ($p<0.05$) .
وجد أن المتوسط ± الخطأ المعياري للمتوسط لمؤشر كتلة الجسم هو 20.6 ± 0.21 و 36.1 ±0.5لذوات الاوزان العادية و اللائي يعانين من السمنة على التوالي. و كان أعلى مع دلالة إحصائية في من يعانين من السمنة ($p<0.01$).
أظهرت الدراسة أن نسبة البدانة هي 39.1 ٪ من المشاركات بإعتبار أن النقطة الفاصلة لمؤشر كتلة الجسم $\leq$ 30كجم/متر^2. بناء على تصنيف المشاركات على اساس نسبة الدهون في الجسم ، الناتج عن قياس سمك ثنية الجلد من أربع نقاط ، ارتفعت نسبة البدانة في المشاركات إلى 51.5 ٪ بإعتبار أن النقطة الفاصلة لنسبة الدهون هي $\leq$ 30 ٪.

في 26,7 ٪ من المشاركات كان محيط الخصراعلى من 88 سم وفي 94.6 ٪ منهن كانت نسبة محيط الخصر لمحيط الحوض اقل من 0.85. في 14.5 ٪ فقط من من يعانين من السمنة كانت نسبة محيط الخصر لمحيط الحوض اكبر من 0.85 ، ويعزى انخفاض نسبة محيط الخصر لمحيط

الحوض إلى زيادة محيط الحوض في المشاركات في الدراسة. وجد أن محيط الحوض لذوات الاوزان العادية و اللائي يعانين من السمنة هو 47.100 ±0.62و 77.124 ± 1.27سم على التوالي .
تم قياس ضغط الدم الإنقباضي و الإنبساطي لجميع المشاركات و كان في نطاقه الطبيعي لجميع المشاركات إلا 21.8 ٪ من من يعانين من السمنة

في هذه الدراسة وجد أن سكر الدم الصائم طبيعي في كل المشاركات .
أظهرت الدراسة أن هناك قيم أعلى لمعالم الدهن في الإناث اللائي يعانين من السمنة بالمقارنة مع ذوات الاوزان الطبيعية. و كان المتوسط ± الخطأ المعياري للمتوسط للكوليسترول الكلي ، كوليسترول - البروتين الدهني العالى الكثافه،و كوليسترول - البروتين الدهني المتدنى الكثافه و الدهون الثلاثية الجلسريدات هو 148.2 ± 3.03، 37.42± 1.36، 92.7 ± 3.33 و 90.79 ± 4.41 مغ / دل على التوالي ، في حين المتوسط ± الخطأ المعياري للمتوسط لنفس هذه المعالم في الإناث ذوات الاوزان الطبيعية كان 136.0 ± 3.23 ،44.0± 1.20 ،76.95 ± 3.43 و 75.51 ± 3.71مغ / دل على التوالي. جميع هذه القيم تقع ضمن النطاق الطبيعي

أظهرت الدراسة ارتفاع بروتين سي التفاعلي في الإناث اللائي يعانين من السمنة بالمقارنة مع ذوات الاوزان الطبيعية ، و كان المتوسط ± الخطأ المعياري للمتوسط 0305 ±0.09و6.256± 0.78 على التوالي و هذا الاختلاف ذو دلالة إحصائية مع($p<0.008$). لم تظهر هذه الدراسة فرق في إنترليوكين - 6. حيث أن المتوسط ± الخطأ المعياري للمتوسط لإنترليوكين - 6 كان 5.16± 0.73و 5.76 ± 0.34 في الإناث اللائي يعانين من السمنة و ذوات الاوزان الطبيعية على التوالي ($p<0.101$)

الخلاصة : أشارت النتائج إلى أن الدهون في من يعانين من السمنة من الإناث تتراكم في الجزء الأسفل من الجسم بدلا من منطقة البطن. كبر محيط الفخذ والحوض تعكس زيادة النسيج الشحمي تحت الجلد ؛ هذه المستودعات غنية نسبيا بخميرة اللايبوبروتين لايباز ومعدل منخفض نسبيا لتحلل الدهن. بالإضافة إلى ذلك ، فإن أغلبية من يعانون من السمنة الإناث المشاركات في الدراسة يتمتعن بقياسات طبيعية لضغط الدم و مستوى سكر الدم الصائم ومعالم الدهون ؛ وفقا لذلك فإنهم يعانون من السمنة المفرطة و لكنهم اصحاء(MHO).

ABBREVIATIONS

4-AAP	4-aminoantipyrine
apo	Apolipoprotein
ATP	Adenosine triphosphate
BIA	Bioelectric Impedance Analysis
BMI	Body mass index
CCK	Cholecystokinin
CETP	Cholesteryl ester transfer protein
ChEH	Cholesterol esterhydrolase
ChOD	Cholesterol oxidase
Co A	Coenzyme A
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
CT	Computerized Tomography
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEXA	Dual Energy X-ray Absorptiometry
EDTA	ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
Fc	Fragment crystallizable
GHRH	Growth hormone releasing hormone
GK	Glycerol kinase
GLP-1	Glucagon-like peptide-1
GOD	Glucose oxidase
GPO	Glycerol phosphate oxidase
HC	Hip circumference

HDL	High-density lipoprotein
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
hs-CRP	High-sensitivity testing for C-reactive protein
ICAM-1	Intercellular adhesion molecule-1
IL-6	Interleukin-6
IOTF	International obesity task force
LDL	Low-density lipoprotein
LRP	LDL-receptor related protein
MHO	Metabolically healthy but obese
MRFIT	Multiple Risk Factor Intervention Trial
MRI	Magnetic Resonance Imaging
mRNA	Messenger ribonucleic acid
MSH	Melanocyte-stimulating hormone
MTP	Microsomal triacylglycerols transfer protein
NHANES II	Second National Health and Nutrition Examination Survey
NPY	Neuropeptide Y
***ob* gene**	Obese gene
PCO_2	Partial pressure of carbon dioxide
PDH	Pyruvate dehydrogenase
POD	Peroxidase
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
SBP	Systolic blood pressure
SES	Socioeconomic status
SNS	Sympathetic nervous system
SR-B1	Scavenger receptor class B1

TAG	Triacylglycerols
ThC	Thigh circumference
TNF-α	Tumor necrosis factor-α
TOBEC	Total body electrical conductivity
TUB	Tubby homolog (mouse)
VLDL	Very-low-density lipoprotein
VMH	Ventromedial hypothalamic
WC	Waist circumference
WHO	World health organization
WHR	Waist hip ratio

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER ONE

Introduction` and Literature review

Obesity constitutes one of the most important public health problems nowadays. It results from deposition of excess fat in the body, caused by ingestion of greater amounts of food that can be used by the body for energy[1]. The excess food, whether fats, carbohydrates, or proteins, is then stored almost entirely as fat in the adipose tissue to be used later as energy. Obesity may be defined functionally as a maladaptive increase in the mass of somatic fat stores. Overweight and obesity adversely affects blood pressure, cholesterol, triacylglycerols (TAG) and insulin resistance. The risks of coronary heart disease, ischemic stroke and type 2 diabetes mellitus increase steadily with increasing body mass index (BMI).

It is important to note that it is not only the amount of the excess fat that is important, but also its distribution within the body which determines the health risks associated with the obesity [1].

1.1. Control of body weight

The maintenance of an adequate body weight is a major determinant of the survival of higher organisms including mammals. Stability of body weight and body composition over long periods of time requires that energy intake matches energy expenditure. In human adults, there are mechanisms that tend to maintain energy intake and energy expenditure in balance. It is important to emphasize that body weight regulation not only requires the maintenance of energy balance, but also nutrient balance must be achieved, i.e., the mixture of fuel oxidized must be adjusted to match the composition of food ingested [2].

Body weight regulation involves various tissues that are composed of proteins, carbohydrates, fats, water, and minerals. Acute changes in body weight can result from alterations in fluid balance, such as dehydration during prolonged exercise without adequate water intake. The mechanisms of water balance are well known and allow adjustment of body fluids within a few hours. Body weight regulation depends mainly on mechanisms that control the utilization and storage of fuel nutrients such as

carbohydrates and lipids, and because proteins and carbohydrates stores in adults body vary relatively little. The body weight regulation mainly concerns adipose tissue mass. Chronic imbalance between fuel nutrients intake and energy expenditure results in changes in adipose tissue mass. Therefore, body weight regulation implies that the adipose tissue mass is "sensed," leading to appropriate responses in individuals who maintain body weight and body composition constant during prolonged periods of time [3].

There is preponderant evidence for the existence of an adipose tissue mass control with signals that come in part from adipose tissue and that act on hypothalamic receptors with effectors in the autonomic nervous system. The control of adipose tissue mass requires a highly integrated and redundant neurohumoral system that minimizes the effects of short-term fluctuations in energy balance [3].

A major breakthrough in obesity research has been the identification of genetic loci (*ob* gene) at which specific mutations cause obesity in mice and rats. The cloning of the *ob* gene and identification of its encoded protein leptin have provided a feedback signaling system reflecting the amount of adipose energy stores [3,4]. The *ob* gene product, leptin acts via hypothalamic receptors to inhibit feeding, increase thermogenesis, and decrease body weight in rodents [4]. Thus, it is clear that body weight is regulated through a feedback regulatory loop with three distinct steps:

1) A sensor that monitors the level of energy.

2) Hypothalamic centers that receive and integrate through leptin receptors the intensity of the signal.

3) Effectors system that influence the determinants of energy balance, i.e., energy intake and energy expenditure.

The afferent limb of the regulatory loop of body weight regulation consists of hormones that are secreted in proportion to body fat mass. Leptin, a protein produced by adipose cells, fulfills this criteria, since its plasma concentration in humans is proportional to body adiposity [4]. The hypothalamic targets are leptin-responsive neurons. The binding of leptin to its receptor alters the expression of several genes producing specific

neuropeptides such as , **[**neuropeptide Y (NPY), agouti-related peptides (AgRP), proopiomelanocortin (POMC) products including α-melanocyte-stimulating hormone (α-MSH) and other melanocortin-4 receptor ligands, corticotropin-releasing hormone (CRH), melanocyte concentrating hormone, orexin, and tubby (TUB)**]** [5] that modulate food intake and energy expenditure [6]. The efferent limb of the regulatory loop is represented by neuronal network containing neurons with specific receptors for the hypothalamic neuropeptides mentioned above. The autonomic nervous system is also implicated in this efferent limb; leptin increases sympathetic nervous system (SNS) activity [7], which mediates its action on energy expenditure, whereas NPY acting on the paraventricular nucleus (PVN). NPY receptors, reduces SNS outflow to brown adipose tissue [8].

When energy stores decrease, due to prolonged nutritional deprivation, one expects a stimulated food-seeking behavior and decreased resting energy expenditure. In contrast, with nutritional abundance, a feature of most developed countries, one observes a high prevalence of obesity; furthermore, the recent increase in the incidence of obesity in many developing countries suggests that the mechanisms of body weight regulation are easily altered when food availability increases. There is evidence that a high-fat diet overrides satiety mechanisms; however, the concomitant decline in physical activity and the modern inactive life-styles are also important factors that parallel the secular trends in obesity [9]. A variety of genetic, dietary, and life-style factors contribute to determine the steady state of weight maintenance at which the daily oxidation of a fuel mix matches the amount and the composition of the nutrients of the diet [2]. Thus it can be concluded that the size of the adipose tissue mass is not under a strict set-point control [10].

Despite the recent advances in the understanding of the physiology of body weight regulation, obesity prevalence is increasing in many countries, which indicates that the prevention of excessive body weight gain and the treatment of obesity have not improved over the last decades. This area of great public health relevance has not yet benefited from the remarkable advances in the understanding of the pathophysiologt is hoped that recent developments in molecular and cellular biology will result in new therapeutic

approaches that not only improve the efficacy of weight loss strategies but that may also reset body weight regulation to a new lower set point [10].

1.1.1. Nutritional balance

Maintenance of a constant body weight and body composition requires that energy and nutrient balances are achieved. The concept of nutrient balance stems from the fact that each of the three macronutrients (carbohydrate, fat, and protein) is either oxidized or stored. The conversion of a nutrient into another for storage does not represent important metabolic pathways [11]. Although it is commonly believed that hepatic *de novo* lipogenesis is a mechanism by which fat accumulation occurs in humans, recent evidence indicates that only a few percent of glucose carbon atoms are converted into fatty acids and leave the liver as very-low-density lipoprotein (VLDL) triacylglycerols in rat [12]. The *de novo* lipogenic response to a high-carbohydrate, low-fat diet is stimulated as compared with a high-fat diet, but the total amount of de novo fatty acids synthesized remains low and does not exceed 12g/day. Furthermore, during carbohydrate overfeeding, the hepatic de novo lipogenesis was found not to exceed 5-10 g fatty acids synthesized per day [13]. De novo lipogenesis may occur during simultaneous lipid oxidation and will not result in net lipid deposition unless the amount of fat synthesized exceeds that of fat oxidized. Net lipogenesis, corresponding to accretion of lipid stores from carbohydrate, can be documented by the presence of respiratory quotients higher than 1.0. Such a net lipogenesis has been observed in humans only during periods of forced massive overfeeding, a condition which does not occur in everyday life. Recent observations indicate that hepatic lipogenesis accounts for only a minor portion of total fat synthesis in these conditions, suggesting that adipose tissue lipogenesis may play an important role [14].

The metabolic responses to dietary carbohydrate and fat differ markedly. Dietary carbohydrate stimulates insulin release, a response which serves to limit the rise in glycemia. The increase in plasma insulin concentration promotes glucose uptake in insulin-sensitive tissues and inhibits hepatic glucose production. Insulin stimulates glucose transport into muscle and glycogen synthesis in both muscle and liver. In addition, insulin decreases the release of free fatty acids from adipose tissue by inhibiting

hormone-sensitive lipase and stimulates triacylglycerol uptake in adipose tissue by activating lipoprotein lipase. The postprandial rise in glycemia and in insulinemia, in combination with a reduced plasma free fatty acids concentration, results in an increase in the proportion of energy derived from carbohydrate oxidation and in a decrease in that derived from fat oxidation in the whole body [15].

Although a high-carbohydrate meal promotes carbohydrate oxidation, by contrast the metabolic responses after a high-fat meal mainly consist in a stimulation of fat storage without stimulation of fat oxidation. Only very high-fat meals induce a slight increase in fat oxidation, the majority of fat intake being stored in adipose tissue. It can be concluded that carbohydrate balance has a priority over fat balance. Furthermore, nitrogen balance tends to be maintained within a few days, even in the presence of changes in the amount of daily protein intake; when the minimum dietary protein requirement is met, the body's protein mass remains stable. There is ample evidence showing that in adult humans, variations in energy balance are reflected by changes in fat balance, the protein and carbohydrate balances being achieved within a few days [2]. The weight gain, which characterizes the development of obesity, mainly results from the accumulation of dietary fat in adipose tissue; the latter is due to the inability to oxidize the total amount of the daily fat intake [15].

The importance of dietary fat in the development of obesity is further emphasized by most epidemiological studies which show a positive association between fat intake and body weight [16]. The fuel mix oxidized may also influence body weight regulation. In certain groups of sedentary individuals, a high insulin sensitivity was associated with subsequent weight gain,[17, 18] indicating an increased carbohydrate oxidation and a reduced lipid oxidation. Thus both excess of fat intake and low fat oxidation are two factors that favor weight gain and therefore the development of obesity. The mechanisms controlling fat oxidation are therefore of great importance in the context of body weight regulation [18].

The enhanced free fatty acid (FFA) oxidation produces an increased acetyl CoA-to-CoA-SH ratio and an augmentation of cytoplasmic citrate concentration. The elevated

concentration of acety- CoA activates pyruvate dehydrogenase kinase, which phosphorylates and thus inhibits pyruvate dehydrogenase (PDH). Glucose catabolism is inhibited at two important steps [19].

1) The increase in cytoplasmic citrate concentration inhibits phosphofructokinase, which results in an increased glucose-6-phosphate concentration; as a consequence, hexokinase is inhibited and finally glucose uptake is impaired.

2) Inhibition of PDH impairs the entry of pyruvate into oxidative metabolism and thus contributes to inhibit glucose oxidation.

In the whole body, the total rate of fat oxidation is dependent on the concentration of plasma free fatty acids [19, 20]. However, the utilization of triacylglycerol deposits in various tissues, such as skeletal muscle, also influences total body fat oxidation. A mechanism that tends to increase total body fat oxidation is the enlargement of the adipose tissue mass. The increased FFA release into the circulation in obese subjects is not a straightforward matter of quantity of adipose tissue. The elevated plasma free fatty acids concentration is most pronounced in abdominal obesity,[21] a condition which is often associated with insulin resistance and hyperinsulinemia.

Body weight eventually reaches a near-constant level in obese individuals in spite of an excess of energy and fat intake. Two homeostatic mechanisms have been described that are related to the composition of the body weight gain (~75% fat and 25% fat free mass):

1) The enlargement of the fat free mass is accompanied by an increase in basal metabolic rate and, therefore, enhanced total energy expenditure [22-24].

2) The increase in the fat mass is accompanied by an enhanced rate of FFA release into the circulation, which contributes to stimulate fat oxidation.

Thus the enhanced fat oxidation observed in obese individuals in the resting state might serve as a lipostatic mechanism in individuals who are gaining weight. This metabolic adaptation eventually allows fat oxidation to rise to a level matching fat intake, thus limiting further weight gain. Studies on the relationship between fat mass and fat oxidation showed that a 10 kg increase in fat mass corresponds to a stimulation of fat oxidation of ~20 g/day [25]. Thus enlargement of body fat serves as a mechanism that

contributes to equilibrate fat balance in individuals with a chronic excess of fat intake [26]. A positive energy balance, particularly due to carbohydrate overfeeding, also stimulates sympathetic activity,[27] a mechanism which may contribute to increase energy expenditure.

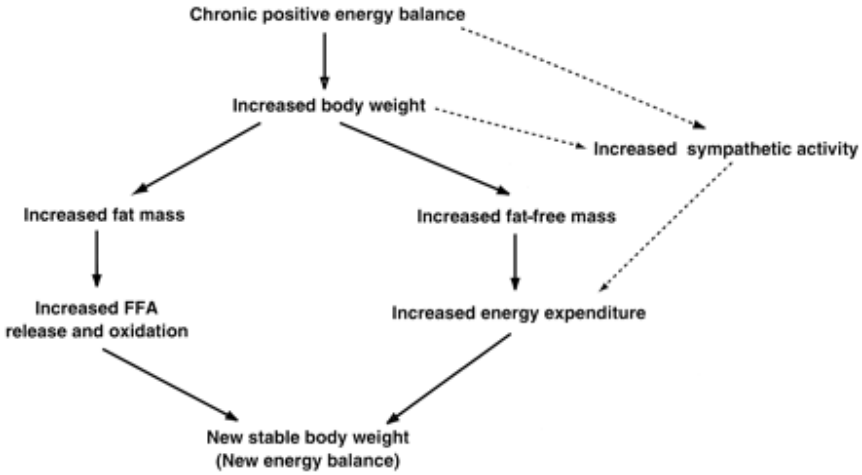


Fig: (1). Metabolic consequences of a chronic positive energy balance on fat mass and fat-free mass. Induced increases in free fatty acid (FFA) release, FFA oxidation, and energy expenditure eventually leads to a new stable body weight, resulting from a new energy balance. Solid arrows, main mechanisms; dashed arrows, mechanisms mainly operating in rodents.

1.1.1.1. Satiation and satiety

Most people who maintain a stable body weight spontaneously adapt their energy intake to a higher rate of energy expenditure through accurate mechanisms of control of food intake. Appetite is a complex phenomenon arising from a sequence of interactions among peripheral and central mechanisms. The gastrointestinal tract contains chemo- and mechanoreceptors that relay the information about its nutrient content to the brain mainly via the vagus nerve [28]. Impairment of appetite or satiety may arise from peripheral or central mechanisms.

The amount of energy ingested over 24 h depends on two major variables: the size of individual meals and the frequency with which meals are ingested. These two variables are regulated by distinct mechanisms. Hunger can be defined as the sensation felt by an individual that drives him to search for and ingest food. This sensation is elicited after a variable period following the absorption of the nutrients ingested with the previous meal. Although its mechanisms remain poorly understood, it has been repeatedly observed that

a slight fall in plasma glucose concentration precedes the initiation of food intake in both rats and humans. After the ingestion of a certain amount of food, a suppression of hunger occurs that will lead to the termination of food intake. This process is referred to as satiation, and the mechanisms that underlie it are the major determinants of meal size. The time of satiation is followed by a period of variable duration that is characterized by the absence of hunger; this is referred to as satiety. Termination of the period of satiety coincides with the resurgence of the feeling of hunger, leading to consumption of the next meal, thus resuming the cycle of food intake [29, 30]. The mechanisms that promote satiation are different from those that determine the duration of satiety; thus meals size and meals frequency are controlled by different factors. Not only the macronutrients composition, size, and caloric density of the meals, but also their organoleptic properties (sight, smell, taste, and texture) play an important role in the determination of satiation. In addition, it has been demonstrated that individuals who were voluntarily overfed or underfed over extended periods of time to achieve significant changes in body weight tend to restore their usual body weight within a period of several weeks or months; such individuals spontaneously reduced or increased their food intake when placed again on an ad libitum diet until their body weight came back to initial values [31]. Thus, in addition to the effects of nutrients ingested with the previous meals, body size and body composition obviously play a more chronic role in the control of food intake. Furthermore, it is evident that, in our modern civilizations, food intake is not invariably the result of hunger. Numerous situations may lead to food or drink consumption as a result of social activities [32]. Alterations of food intake may also result from complex psychodynamic stimuli, as is probably the case in anorexia nervosa and bulimia nervosa.

1.1.1.2. Hypothalamic and brain stem centers

It has been recognized for several decades that hypothalamic and brain stem centers control food intake and energy expenditure in animals and in humans. In the rat, it was observed that electrical stimulation of the lateral hypothalamic area triggered food and drink intake while electrical stimulation of the ventromedial area induced termination of food intake [33-35]. It was further observed that physical or chemical lesions of the ventromedial area of the hypothalamus in rats led to the development of obesity and

insulin resistance Such lesions induced marked alterations of the feeding behavior, in such a way that affected animals consumed a large excess of calories when given palatable food in sufficient amounts, but failed to actively search for food during food deprivation. They also displayed neuroendocrine abnormalities; lesions of the ventromedial hypothalamic (VMH) area were characterized by early hyperinsulinemia, which was mediated by an increased vagal activity and could be prevented by vagotomy, and a decreased overall sympathetic activity and brown adipose tissue thermogenesis [36].

Nuclei within the lower brain stem integrate and relay information between peripheral autonomic/endocrine organs and other forebrain structures. Nuclei in the pars-midbrain and the thalamus interpret this information in relation to the sensory properties of food. Hypothalamic nuclei respond to neural inputs as well as to circulating hormones and substrates. Finally, forebrain nuclei such as the amygdala and the frontal cortex are involved in the aversive or positive aspects of food intake. Various inputs, including neural inputs from the vagus nerve ormones, and possibly substrate concentration changes inform these regulatory centers on the metabolic status of the body [37].

Other central effectors, galanin, catecholamines, and opioid peptides, have been shown to exert potent antagonist actions on food intake and more particularly on fat intake [38]. More recently, glucagon-like peptide-1 (GLP-1) has also been shown to be a potent inhibitor of food intake [39]. Other factors, among which glucocorticoids (by acting on the mineralocorticoid type receptor within the CNS) and growth hormone releasing hormone (GHRH) potentiate food intake, but their role in body weight regulation remains uncertain [39-40].

Signals from metabolic origin may also contribute to the sensation of satiety in mammals such as the degree of oxidative metabolism of glucose and FFA in the liver. It has been shown that inhibition of fat oxidation by methyl palmoxirate or 2-mercaptoacetate causes an increase in feeding [41, 42] Suppression of appetite resulting from this mechanism is, however, not necessarily due to the ingestion of fat, since fuels derived from internal adipose stores, as it occurs during fasting, may also provide FFA for oxidative liver metabolism. There has been a large interest in the search of peripheral satiety signals

arising from adipose tissue that could reflect the degree of repletion of fat stores, and therefore be candidates for feedback signals in a regulatory loop. Substances such as adipsin, and oleoyl-estrone are produced by adipose cells, but their role in appetite control is uncertain [43-45].

1.1.1.3. Effects of nutrients on food intake

The influence of nutrients on subsequent food intake has been extensively studied by covertly altering the food composition or energy content of a meal and observing the changes in the subsequent food composition of the next meal. It has been generally observed that a selective deficit in one of the major macronutrients did not trigger a specific increase of the intake of this specific macronutrient, but rather a compensatory ingestion of an equivalent number of calories from a mixed diet [29, 30, 46, 47]. In contrast, excessive intake of nutrients generally decreases subsequent food intake.

There is a hierarchy regarding the ability of the various macronutrients to suppress subsequent food intake. Proteins display the most potent effect to delay subsequent nutrient ingestion. Carbohydrates, whether administered orally or parenterally, are also able to significantly increase the early satiety period and to decrease the amount of food ingested at the next meal. Lipids appear to have less potent satiating effects. Of interest, intravenous infusion of lipid emulsion failed to alter voluntary food intake, while intraduodenal administration of lipid was effective [48]. This indicates that gut factors may be responsible for the satiating effects of lipids. Stimulation of cholecystokinin (CCK) secretion by enteral lipid is likely to play a significant role in this regard. The role of CCK as a hormone that mediates satiation and early-phase satiety has been recently emphasized [49]. The intake of protein and fat stimulates the release of CCK from cells in the mucosa of the upper small intestine. This hormone activates CCK-A receptors in the pyloric region of the stomach; the signal is then transmitted via vagal afferents to the nucleus of the tractus solitarius, where it is relayed to the PVN and to the VMH [49]. Another peptide, enterostatin, appears to selectively reduce intake of a high-fat diet [50].

1.1.1.4. Influence of the increasing proportion of dietary fat on composition of food intake

Diet composition differs markedly among countries and cultures. Traditional African diets for instance are characterized by a high content in carbohydrate and fibers, and it is interesting to note that obesity is virtually absent in societies eating this type of food. In contrast, in industrialized countries where obesity is highly prevalent, fat may represent 40% or more of total calories ingested. Such a trend toward increasing both dietary fat and the prevalence of obesity has also been reported among people of the high socioeconomic classes in many developing countries over the past decade [51, 52] Although such dietary changes are usually paralleled by significant reduction in physical activity, it raises the suspicion that dietary composition, and in particular the increasing proportion of fat, may be a major determinant of the energy content of the daily food intake.

This hypothesis is indeed supported by several observations. First, the effect of altering the fat content of the meals was monitored in healthy lean human subjects. It was observed that when food items with a high fat content were presented, subjects ate 30% more calories per day compared with what they ate when presented with food items with a higher carbohydrate content [53, 54]. Interestingly, this excessive amount of calories ingested on a high-fat diet was consumed as a smaller volume, as well as a smaller weight of food due to the higher energy density of high-fat foods. Second, it has been reported in several surveys that the diet composition of obese subjects contain a higher proportion of fat than that of lean individuals. This observation strongly suggests that a habitual high dietary fat content may lead individuals to obesity due to the lower satiating effect of fat compared with carbohydrates [16, 47, 55, 56]. Third, the observation that obese subjects lose weight when placed on an ad libitum high-carbohydrate diet further supports the hypothesis that high-carbohydrate, low-fat diets are more satiating than high-fat diets [57].

1.2. Measurement of obesity

Obesity specifically refers to an excess amount of body fat, defined as body mass index (BMI) of greater than or equal to 30kg/m^2. To most people, obesity means overweight, however health professionals define "overweight" as an excess amout of body weight that includes muscles, bone, fat and water [58].

There are various methods to measure and estimate body composition and the distribution of fat. These range from the simple, useful and practical anthropometric measurements such as weight and height, waist circumference, waist /hip ratio (WHR), skin fold thickness to the more sophisticated measurements such as Hydrodensitometry, Magnetic Resonance Imaging (MRI), Computerized Tomography (CT), Dual Energy X-ray Absorptiometry (DEXA), Bioelectric Impedance Analysis (BIA) and Air Displacement Plethysmography used in research [58, 59].

1.2.1 Body mass index (BMI)

The BMI which is calculated from simple anthropometric measurements, is the most widely used population index for the classification of obesity and the risks associated with it (Table 1.1) [60]. It provides a more accurate measure of total body fat than assessment of body weight alone. However, it does not address the distribution of fat i.e. android obesity, or abdominal adiposity, and gynoid or gluto-femoral fat adiposity. BMI is an index of weight for height and is calculated as the weight in kilograms divided by the square of the height in meters (Kg/m^2). It has been long realized that BMI is a predictor of the morbidity and mortality associated with, the chronic diseases: such as type 2 diabetes CVD disease and stroke [60, 61]. Raised BMI also increases the risks of cancer of the breast, colon, prostate, endometrium, kidney and gallbladder. Although mechanisms that trigger these increased cancer risks are not fully understood, they may relate to obesity-induced hormonal changes. Chronic overweight and obesity also contribute significantly to osteoarthritis, a major cause of disability in adults [62]. It has also been shown that the health risk increases in a graded fashion when moving from the normal-weight through to obese BMI category,[61] and that within each BMI category men and women with high waist circumference (WC) values are at a greater health risk than those with normal WC values. Thus, it is assumed that the BMI and WC have independent effects on obesity related co-morbidities [63].

1.2.1.1. Limitations of the BMI

The BMI does not distinguish between weight associated with fat and weight associated with muscle mass. Hence, factors such as body build and proportions are not taken into account and therefore the same BMI may reflect variations in body composition other

than in body fat. Hence, BMI can therefore not be used in a muscular person because as much as the weight of the muscular individual will be high, their fat mass may be low so classifying them as obese would be incorrect. Moreover, BMI does not address the type of obesity, such as android or gynoid, are known to be better determinants of current and future pathology of co-morbid diseases. Thus, although the BMI is a useful source of primary information, it should best be interpreted in combination with other assessment methods of body composition [63].

Table. (1) The classification of overweight and obesity in adults according to BMI

Classification	BMI (kg/m²)	Risk of co-morbidities
Underweight	<18.5	Low; but risk of clinical complications increases
Normal Range	18.5 – 24.9	Average
Overweight	25 – 29.9	Mildly increased
Obese	≥30	
Obese Class I	30 – 34.9	Moderate
Obese class II	35 – 39.9	Severe
Obese class III	>40	Very Severe

BMI: Body mass index

1.2.2. Waist circumference (WC)

Abdominal obesity, assessed by waist circumference (WC) predicts obesity related health risks [63, 64]. This is a simple measurement, which correlates closely with the BMI and waist hip ratio (WHR). WC has been shown to be a good indicator of intra-abdominal fat mass in adults and was associated with increased risk for chronic diseases. It is measured at the midpoint between the iliac crest and the lower border of the rib cage. It is an appropriate index of intra-abdominal fat mass and total body fat and is strongly correlated with computerized tomography (CT) scan. The waist circumference is mainly used as an initial screening tool. However, due to difference across populations regarding level of risk associated with waist circumferences, global cut-off points have not yet been developed. Changes in the waist circumference suggest changes in risk factors for chronic disease, especially cardiovascular disease [63, 65]. High WC values have been associated

with an increased risk of hypertension, diabetes, dyslipideamia, and metabolic syndrome. The National Institute of Health cut off points for WC help to identify those at increased health risk within the normal weight, and overweight and class I obese BMI categories [61].

1.2.3. Waist hip ratio (WHR)

The location of fat in the body is as important as the amount of fat stored. Indeed, abdominal fat mass is considered to be of the essence in relation to the complications of obesity [61].

Table. (2): Waist circumference in men and women associated with increased risk for chronic diseases of lifestyle

Disease Risk	Women	Men
Desired weight	< 80 cm	< 94 cm
Increased health risk, aim to loose weight	80- 87.9	94 – 101.9
High health risk, must loose weight	≥ 88 cm	≥ 102 cm

The WHR is an accepted method of identifying individuals at increased risk from obesity-related illness due to abdominal fat accumulation. A high waist in females predicts cardiovascular mortality in some populations, and to some extent is independent of the degree of obesity as assessed by the BMI [58]. Given the difficulty of using the age adjusted associations in clinical setting, the available evidence suggests that given appropriate cut-off points, WHR is the most useful measure of obesity in the identification of individuals at risk of CVD (Table 1.2) [64].

1.2.4. Skin-fold thickness

Over half the fat in the body is deposited under the skin, and the percentage increases with increasing weight. Skin fold measurements obtained from different sites of the body can be a reliable estimate of obesity and fat distribution. Calipers are used to measure a pinch of subcutaneous fat from several areas of the body, the triceps, trunk, sub scapular area, and thigh, and are compared to age, race, and sex-matched controls to calculate percent body fat. Because the amount of fat distributed differs from place to place in the body, some investigators have suggested using the sum of skin folds from different areas

to asses the total body fat. Durnin and Womersley derived tables for instance, use the sum of four skin folds (biceps, triceps, subscapular, and suprailiac) and relate them to the fat content of the body. If done correctly, this method is useful in the assessment obesity, but inter-observer variability may be high, making reproducibility difficult. Even though skin fold calipers are inexpensive and mobile, it requires some training to perform measurements [61].

1.2.5. Bioelectric impedance analysis

Bioelectric impedance analysis (BIA) estimates the percentage of body fat by relying on the association of conductivity of tissue fluid and electrolyte content. A small alternating current is applied, which is primarily conducted by body water and dissolved electrolytes. This method will determine the total body water used to predict body fat percentage. It is fairly reliable, but less accurate in obese children. This non invasive method, requires no special training to perform or interpret results, and is relatively inexpensive. Overall, the widespread utility of its use in children is still in question, but it can be a useful tool to estimate body fat [61].

1.2.6. Ultrasound

Ultrasound is a cheap, easy to use method of determining body fat percentage, but tends to measure only subcutaneous fat, which may over or underestimates total body fat and obesity. It is used primarily in the research setting [61].

1.2.7. Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DEXA) estimates fat-free mass as well as body fat. Using differential attenuation of x-rays passing through tissue. DEXA differentiates fat from lean tissue, it gives accurate results with great reproducibility, but is very expensive and takes time to perform. In addition, there is radiation exposure, which can be important when serial measurements are taken in obese children. However, even thought it is used mainly as a research tool, it is becoming widely used in clinical settings [61].

1.2.8. Computed tomography and magnetic resonance imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are excellent tools to determine the fat and lean mass of humans (e.g. body distribution). Both are performed

relatively quickly, and provide whole body as well as regional composition. Unfortunately, like DEXA, CT is expensive, involves radiation exposure, and require trained technicians. Thus, as the case of DEXA, it is used primarily for research purposes [61].

1.2.9. Total body electrical conductivity

Total body electrical conductivity (TOBEC) is a quick and accurate method of measuring body composition coil, which generate a magnetic field. The TOBEC number is generated by the extent of disruption of the field by electrolytes (found in the highest proportion in fat free mass) that are used to calculate adiposity. This method for determining fat and fat free mass is quick and does not involve radiation but is rather expensive [61].

1.2.10. Air displacement plethysmography (Bod-pody composition system)

Air displacement plethysmography is very accurate method of determining body fat. Similar to hydrostatic weighing, the Bod-Pod chamber uses air pressure differences to measure body density, and therefore body fat. This method is suitable for adults, but underestimates body fat in children. Its cost limits the use in a clinical setting [61].

1.2.11. Deuterium

This substance is used to measures body water in order to calculate body fat percentages. Deuterium is not radioactive, and measures total body water. The method is not commonly used outside research field because of the equipment needed, time required, and overall cost [61].

1.3. Prevalence and trends of obesity

The report of the WHO consultation on obesity in 1997 indicates that the prevalence of overweight and obesity is rising to epidemic proportions worldwide in both the developed and developing countries, and in both adults and children. It is predicted that the health consequences and costs of this trend will continue to increase as overweight and obesity among children is increasing worldwide. It has been suggested that obesity should be viewed as a chronic disease. Urban populations showed a higher prevalence of obesity

than rural populations with women having higher prevalence of obesity compared to their male counterparts who show a higher prevalence of overweight [60]. The health, economic and psychosocial consequences of the increasing incidence of obesity are projected to be substantial. In the some reports, approximately 58% of the global diabetes mellitus cases, and 21% of certain cancers were attributable to BMI above 21 kg/m^2. It also accounted for about 13% of deaths in Europe and America, but less than 3% in Africa [66].

In 1995, there were an estimated 200 million obese adults worldwide and another 18 million children under-five classified as overweight. In the year 2000, the number of obese adults had increased to over 300 million. Contrary to conventional wisdom, the obesity epidemic is not restricted to industrialized societies; in developing countries, it is estimated that over 115 million people suffer from obesity-related disorders. Generally, although men may have higher rates of overweight, women have higher rates of obesity. For both, obesity poses a major risk for serious diet-related non-communicable diseases, including diabetes mellitus, cardiovascular disease, hypertension and stroke, in addition to certain forms of cancer. The health consequences range from increased risk of premature death to serious chronic conditions that reduce the overall quality of life [66].

The global prevalence of obesity is now estimated to be 8.2%, significantly higher than the global prevalence of underweight (BMI < 17) which is 5.1% [67]. In the adult population, the prevalence of obesity has increased from 14.5% in 1976-1980 to 22.5% in 1994-1998 .The WHO estimates that adult obesity has increased by 50% between 1995 and 2000, and there are 300 million people affected worldwide. In less developed countries, obesity is usually prevalent among those with higher socio-economic status, it is more frequent in urban areas, and is considered as sign of wealth. In developed countries, on the contrary, prevalence of obesity is high among the poor [68, 69].

1.4. Factors associated with BMI and obesity

Obesity arises from the interaction between genes, environment and behavior. The prevalence of obesity has increased worldwide during the last few decades, while our genes have hardly changed at all [70]. The genetic background of most people is likely not equipped to handle the current abundance of food and a sedentary lifestyle [71]. Thus, the

environment has been suggested to promote obesity-causing behaviors [72]. Nevertheless, little is known about factors that may explain the obesity epidemic or the large differences between populations in the distribution of BMI and the prevalence of obesity [73, 74].

1.4.1 Classification of factors associated with BMI and obesity

1.4.1.1. Demographic factors

1.4.1.1.1. Gender

Women generally have a higher prevalence of obesity (BMI ≥30 kg/m^2), especially after the age of 50 years, whereas men usually have a higher prevalence of overweight (BMI 25-29.9 kg/m^2).[75, 76] Men have more skeletal muscle than women, both in absolute terms and relative to body mass. These differences have been found to be greater in the upper body [77].

1.4.1.1.2. Age

Increase in BMI with age has been documented in several cross-sectional studies.[75, 78-80] The older the subjects, the higher the mean BMI and the prevalence of obesity in both men and women, at least up to the age of 50-60 years [78, 80]. The increase of BMI with age in women tends to continue longer than in men [80, 81]. In addition to cross-sectional studies, the few longitudinal studies support the finding that people generally gain weight as they become older, with 60 years of age typically marking a turning-point [82-86].

1.4.1.1.3. Ethnicity

The prevalence of obesity has been shown to vary across ethnic groups [75]. These differences have been suggested to be partly due to a genetic predisposition to obesity, which becomes apparent especially when individuals are exposed to an affluent lifestyle [74]. When assessing the level of obesity and comparing populations based on BMI, the validity of the BMI cut-off points for obesity may differ for different ethnic groups. Although in some studies no difference has been found between BMI and body fat in different ethnic groups,[87] the majority of studies and a recent meta analysis confirm that the relationship between body fat and BMI varies across ethnic groups [88]. Thus, variations in BMI between ethnic groups should be interpreted with caution [74].

1.4.1.2. Sociocultural factors: education and family situation

1.4.1.2.1. Educational level

The effect of the socioeconomic status (SES)on obesity is well recognized [89]. Especially in women, there has been a strong inverse association between obesity and socioeconomic status, as assessed by educational level [90,91]. The WHO MONICA study showed that the level of education is inversely associated with BMI in women, and the difference between the highest and lowest educational classes is ranging from –3.3 to 0.4 kg/m^2. This association was less consistent in men, although an inverse association was observed in about half of the populations in the 1990s [92].

1.4.1.2.2. Marital status

Marital status has been found to be associated with BMI and obesity, although this relationship is not well established. Several, but not all cross-sectional studies have shown that married or cohabiting individuals had a higher BMI than single individuals. In a few longitudinal studies, the BMI of those who got married during the follow-up period increased more than those retaining the same marital status as at the beginning of the study [93, 94].

1.4.1.2.3. Number of children

Pregnancy has been suggested to ontribute to development of obesity in women [74]. Parity has been observed to be positively associated with BMI in several, [74, 95] but not all [96] cross-sectional studies. In most longitudinal studies, parity has been identified as a predictor of weight gain [97]. The average weight gain associated with pregnancy appears, however, to be quite modest after controlling for age, which has been identified as a much stronger determinant of BMI increase [35,98,99]. The effect of pregnancy on body weight may be due to environmental factors rather than being purely biological. This is supported by findings in which postpartum weight retention has been shown to be more affected by a change in lifestyle during and especially after pregnancy than before pregnancy, both in the general population, [100] and in obese women [101].

1.4.1.3. Dietary intake and physical activity

1.4.1.3.1. Food choices and dietary intake

Nutrition is of critical importance in establishing a positive energy balance. Of the nutritional factors related to obesity, dietary fat intake is widely believed to be the primary determinant of body fat [102]. High-fat diets have been suggested to promote obesity by increasing energy intake, and further increasing the likelihood of a positive energy balance and weight gain [103]. This has been proposed to be due to the greater flavour and palatability of high-fat foods and their high-energy density,[104] together with the weak effect of fat on satiation [105].

Epidemiological studies, however, evidence for a high-fat diet promoting a positive energy balance and development of obesity is not definitive [106]. As reviewed by Lissner and Heitmann (1995),[16] most of the cross-sectional studies have shown positive associations between the percentage of dietary fat and BMI. Some recent studies support this finding, [107] although in other studies this association has been found in men only. [81] Interestingly, an inverse association was reported in one study which shows that women with a higher BMI had a lower fat intake than women with a lower BMI [108].

1.4.1.3.2. Physical activity

Physical activity has three main components: occupational work, household chores and leisure-time physical activity [74]. Physical activity has been shown to be inversely associated with BMI in numerous cross-sectional studies [109]. Obese subjects have been observed to be physically less active than the non-obese [110]. However, in some studies, no association between physical activity and BMI,[111] but an inverse association has been observed only in women [112].

Regardless of physical activity pattern, subjects who reported watching TV more than four hours daily were twice as likely to be overweight than subjects watching TV less than one hour per day [113]. Hours of TV was also positively associated with BMI [114]. Similarly, subjects spending more than 35 hours a week of their leisure time sitting down were 1.6 times more likely to be obese than subjects who spent less than 15 hours per week sitting down [109].

1.5. Pathophysiology of obesity

Ample evidence suggests that obesity increases both morbidity and mortality risks and that fat distribution is important as an independent marker of risk. In spite of controversy about the strength of the independent effect of obesity on health and mortality and its effect on other biological factors, there is little doubt that obesity has huge negative impact on health. It is difficult, however, to assign a specific threshold at which health risks begin. The increase in mortality rates in relation to relative weight increase is steeper in men and women younger than 50 years than in older persons. Moreover the association of the increase in mortality rate with the duration of obesity is also steeper. This observation suggests that a particular effort should be made to prevent weight gain in younger patients [115]. Because little evidence of health risk associated with obesity has been shown at lower weight when adjustments are made for smoking and concurrent disease [115].

1.5.1. Effect of fat distribution

The relation between regional fat distribution and health status has come to the forefront in the last decade. Several prospective, longitudinal studies have shown positive association between increased abdominal fat and overall mortality rate [116, 117]. The issue has been complicated by the fact that these studies have been done by measuring upper body (or central) fat only by anthropometric measures (waist-hip or skinfold thickness ratios). Although precise methods for measuring visceral fat exist, they are costly, invasive, and time-consuming. However, It has become clear that visceral fat, rather than central fat, is related to disease risk. [118].

Central fat deposition is associated with increased risk for coronary heart disease. The relative risk of death was increased progressively with increasing body fat. In addition, cross-sectional studies have associated insulin resistance, hyperinsulinemia, and frank diabetes mellitus with increased abdominal fat [116, 117, 119].

Regional fat distribution has also been related to elevation of serum triacylglycerols levels and a decrease in HDL cholesterol. In addition, increased abdominal fat has also been related to stroke [120].

1.5.2. Specific health consequences

Obesity is associated with an increased risk for insulin resistance, hypertension, dyslipidemia, cardiovascular disease, non-insulin-dependent diabetes mellitus, gallstones and cholecystitis, respiratory dysfunction, and certain forms of cancer [117].

1.5.2.1. Coronary heart disease

Coronary heart disease morbidity is defined as nonfatal myocardial infarction and angina pectoris. The role of obesity on coronary heart disease morbidity and mortality has been widely debated. This debate has been fueled by two issues; first, obesity can enhance other risk factors such as high blood pressure, high blood cholesterol and triacylglycerols levels, low HDL cholesterol, and insulin resistance with hyperinsulinemia that cause coronary heart disease morbidity; second, obesity has been shown to increase coronary heart disease risk independently. This later point has been more controversial because, longer periods of follow-up in longitudinal studies has been necessary to determine significant associations [1]. However, because most studies about obesity and CVD investigated men, a recent longitudinal study in women has received great interest. This study followed 115886 U.S. women who were 30 to 55 years old and who were free of coronary heart disease, stroke, and cancer. A higher BMI was positively associated with the occurrence of each category of coronary heart disease. When different categories of CVD were combined and adjusted for age and smoking status, relative risk for nonfatal myocardial infarction and fatal coronary heart disease went from 1.0 for a BMI of less than 21, to 1.3 for a BMI of 21 to less than 23, to 1.3 for a BMI of 23 to less than 25, to 1.8 for a BMI of 25 to less than 29, and to 3.3 for a BMI greater than 29 [121].

1.5.2.2. Hyperlipidemia

Blood lipid levels are often abnormal in obese persons. Higher levels of high-density lipoprotein (HDL) cholesterol, has been clearly associated with decreased risk of coronary heart disease. HDL is low in obese persons [122,123]. Total and low-density lipoprotein (LDL) cholesterol levels, have been found normal or elevated in obese compared with lean persons [124, 125]. It was also found that, every 10% increase in relative weight was associated with an increase in plasma cholesterol of 12 mg/dL. Because HDL

cholesterol is low and LDL cholesterol is normal or high, the ratio of HDL to LDL cholesterol is generally low, leading to greater atherogenic risk [126].

The Second National Health and Nutrition Examination Survey in the United States (NHANES II), defined hypercholesterolemia as a value at or above 6.47 mmol/L (a clear risk); the relative risk for having such a level was 1.5 times greater in obese than in nonobese persons [127]. In a cross-sectional study, it has been shown that men younger than 39 years had total cholesterol levels that correlated with relative weight, but no such correlation was found for men older than 40 years [124]. A report of a random sample of Neapolitan men and women found higher total cholesterol levels to be associated with increasing BMI [128]. This effect was more pronounced in men. Triacylglycerols have generally been found higher in obese compared with lean persons [129]. Elevated triacylglycerols levels have been associated with weight gain. Increased free fatty acid in blood from enhanced lipolytic activity on one hand and hyperinsulinemia independently enhance the formation of VLDL in the liver [130]. Also, because lipoprotein lipase activity is decreased dung obesity, a decreased clearance of triacylglycerols from circulation also occurs [131].

1.5.2.3. Hypertension

The association between hypertension and obesity is well recognized. Cross-sectional studies have shown that obese persons have a greater risk of high blood pressure than do lean persons [132].

A cross-sectional study conducted from 1976 to 1980 on a representative sample of U.S. residents within The Second National Health and Nutrition Examination Survey in the United States (NHANES II), showed that the prevalence of hypertension among overweight adults was 2.9 times that among non overweight adults [127]. The risk ratio in persons between 20 to 44 years old was 5.6 times greater than that in persons 45 to 74 years old, a figure which was two times higher than that for non overweight adults [133].

Other studies have shown that those persons who are 20% or more overweight have a prevalence of hypertension that is twice that among persons of normal weight [134]. In the

Western World, about one third of cases of hypertension are thought to be due to obesity, and in men younger than 45 years this figure may reach 60% [135]. The reason for the association between elevated blood pressure and increased weight is unclear. A possible cause is a decreased renal filtration surface [136]. Plasma renin activity has also been reported to be elevated in obese persons with hypertension. However, the increased risk of hypertension also leads to an increased risk of stroke [137].

1.5.2.4. Diabetes mellitus

The association between the average weight of population groups and the increased prevalence of non-insulin-dependent diabetes has been repeatedly observed [138, 139]. The risk for diabetes has been reported to be about twofold in mildly obese, fivefold in moderately obese, and 10-fold in severely obese persons [140]. The duration of obesity is a more important determinant of the risk of developing type 2 diabetes mellitus. In cross-sectional studies, obesity has been shown to be associated with an increased prevalence of type 2 diabetes mellitus in both men and women [141]. It has also been found that the overall relative risk of having diabetes was 2.9 times higher for obese persons who are 20 to 75 years old. Moreover, the risk of developing diabetes also increases with age,[127] if a family history of diabetes is present, and if the obesity is concentrated centrally. A prospective study in Scandinavia showed that moderate obesity was associated with a 10-fold increase in the risk of diabetes. This risk increases steeply as obesity become severe [142].

1.5.2.5. Gallbladder disease

Increasing weight is associated with a greater prevalence of gallbladder disease in both cross-sectional and longitudinal studies. Gallstones occur three or four times more often in obese than in non obese persons. The prevalence of gallbladder disease increases with age and with increasing obesity [143]. Women are particularly at higher risk, as shown in the longitudinal prospective studies. Women with a BMI greater than 30 kg/m^2 had a yearly symptomatic gallstone incidence rate of more than 1%, and those with a BMI greater than 45 had a rate of approximately 2% [144]. The gallstones associated with obesity are composed primarily of cholesterol. The explanation for this increase in

prevalence of gallbladder disease with obesity is due to the cholesterol supersaturation of the bile. In addition, greater gallbladder stasis occurs as obesity occurs [145].

1.5.2.6. Respiratory disease

Obesity affects respiratory function. Increased fat in the chest wall and abdomen reduces lung volume, alters the respiratory pattern, and causes a decreased compliance of the respiratory system. Vital capacity and total lung capacity are frequently diminished. In more severe obesity, a ventilation-perfusion abnormality occurs, which is characterized by hypoxia but normal arterial PCO_2 As the severity of obesity increases, sleep apnea occurs with greater frequency. This condition may be obstructive, due to a combination of excess fatty tissue and increased relaxation of the pharyngeal and glossus muscles,[146] or central, due to abnormal control of breathing, or a combination of the two. The full-blown obesity-hypoventilation syndrome is associated with depression of hypercapnic and hypoxic respiratory drives, irregular breathing, frequent apneic periods with resultant severe hypoxia, and daytime somnolence. Cor pulmonale may also occur [147].

1.5.2.7. Cancer

A prospective American Cancer Society study, which followed 750 000 individuals for 12 years and found that the mortality rate for cancer in men who were 40% or more overweight was 1.33. The corresponding figure for women was 1.55. Overweight men had significantly higher mortality rate for colorectal and prostate cancers, and overweight women had significantly higher rates of endometrial, gallbladder, cervical, ovarian, and breast cancers [148]. In longitudinal, prospective studies, the association of obesity with increased mortality rate from breast cancer has been observed in postmenopausal women, and several other studies have generally supported this observation. However, it has been difficult to differentiate the effect of diet from the effect of obesity. In some types of cancer, such as those of the colon and breast, further study may be needed to determine whether obesity simply reflects the diet of the individual patient or whether it is the diet (high fat, high calorie) rather than the degree of obesity that creates the important association [149, 150].

1.5.2.8. Gout

The effect of obesity on uric acid levels shows a sexual dimorphism. In a Canadian Health Survey, the percentage of men with uric acid levels higher than 416 μmol/L increased from 7% to 31% as the BMI increased from 21 to 31. Women were not affected until they reached a BMI greater than 31with a prevalence of 7%. In other surveys, correlations between the level of uric acid and weight repeatedly have been reported [143, 151].

1.5.2.9. Arthritis

Increased prevalence of osteoarthritis has been associated with obesity in several studies. As weight increased in men and women, the prevalence of osteoarthritis increased from 0.75% to 1.45% in men and from 0.4% to 1.45% in women [152].

1.6. Body fats

1.6.1. Importance of assessing abdominal fat

Abdominal fat, or adipose tissue that is centrally distributed between the thorax and pelvis, is out of proportion to total body fat and is an independent predictor of risk factor of morbidity. Abdominal adiposity is associated with greater health risks than peripheral adiposity, e.g., the gluteal-femoral area [153]

Abdominal fat is distributed in three compartments: visceral, retroperitoneal, and subcutaneous. While the relative contributions of these different compartments abdominal fat to overall risk is uncertain several studies indicate that the visceral fat is the most strongly correlated with risk of cardiovascular disease. Other authors indicate that the subcutaneous fat is mostly correlated with insulin resistance. Nonetheless, the presence of increased total abdominal fat appears to be an independent risk predictor when the BMI is not markedly increased. For this reason, waist or abdominal circumference and BMI should be measured for initial assessment of obesity and as a guide to the efficacy of weight loss [154].

1.6.2. Biochemical properties and functions of fats

Lipids are heterogeneous groups of organic compounds comprising fats, and waxes, in addition to other substances. Lipids are one of the chief structural components of living cells. Ninety-five percent of lipids in plasma are carried in lipoproteins. Lipoproteins are small particles that contain triacylglycerols (160 mg/dl), cholesterol (180 mg/dl), phospholipids (160 mg/dl), and proteins (200mg/dl). Lipoproteins are classified into four major types depending on their density as measured in the ultracentrifuge which may reflect the ratio of lipids to proteins. The types of lipoproteins are: high-density (HDL); low-density (LDL); very low-density (VLDL); and chylomicrons [155].

1.6.2.1. Chylomicron

Chylomicrons are large lipoprotein particles that transport dietary lipids from the intestines to other locations in the body. Chylomicrons are one of the four major groups of lipoproteins which enable fats and cholesterol to move within the water based solution of the blood stream. Chylomicrons transport exogenous lipids to liver, adipose, cardiac and skeletal muscle tissue where their triacylglycerols components are unloaded by the activity of lipoprotein lipase. Consequently chylomicron remnants which are left over will taken up by the liver [156].

1.6.2.2. Very low-density lipoproteins (VLDLs)

VLDLs contain high concentrations of triacylglycerols, moderate concentrations of serum cholesterol (10-15 percent) and phospholipids. Intermediate-density lipoproteins are very low-density lipoproteins from which a share of the triacylglycerols has been removed, so the concentrations of cholesterol and phospholipids are increased [156].

1.6.2.3. Low-density lipoproteins (LDL)

LDL is made from intermediate-density lipoproteins by removal of most triacylglycerols leaving a high concentration of cholesterol and moderately high concentration of phospholipids. Low-density lipoproteins (LDL) contain most of the cholesterol (60-70 %) in the blood. It carries cholesterol to the tissues of the body. High levels of LDLs directly correlate with increased risk of cardiovascular disease [156].

1.6.2.4. High-density liproproteins (HDL)

Contain a high concentration of protein (approximately 50 percent) but much smaller amounts of cholesterol and phospholipids. These lipoproteins carry cholesterol away from body cells and tissues to the liver to be excreted from the body. The higher the serum HDL level, the better. Low levels of HDL increase the risk of heart disease. Normally, the HDL component of total body cholesterol is 20-30% [156].

1.6.2.5. Phospholipids

Synthesized in all cells of the body. Although, when the need arises, large quantities are formed in the liver and in the intestinal epithelial cells during lipid absorption from the gut. Phospolipids always contain one or more fatty acid molecules and one phosphoric acid radical, and they usually contain a nitrogenous base. The major types are lecithins, cephalins, and spingomyelin. While these compounds vary in their chemical structures, their physical properties are similar. They are all lipid-soluble, transported as lipoproteins, and used throughout the body for various structural purposes [156].

1.6.2.6. Cholesterol

Cholesterol is a soft waxy substance which is highly fat-soluble and only slightly soluble in water, it is present in diet of most people (called exogenous) and it can be absorbed slowly into the intestinal lymph system. Cholesterol is formed in great quantities by cells of the body (endogenous production). Endogenous cholesterol that circulates in lipoproteins of the plasma is formed in the liver. All other cells form cholesterol. It is a major constituent of cell membranes and used by the body to produce hormones, bile acid, and Vitamin D [157]. The basic structure of cholesterol is a sterol nucleous synthesized almost entirely from acetyl-CoA. In turn the sterol nucleus can be modified by side chains to form ,cholesterol, cholic acid and many important steroid hormones secreted by the adrenal cortex -adrenocortical hormones: the ovaries- progesterone and estrogen; and the testes-testosterone [158].

1.6.3. Fat digestion and absorption

Lipids were thought to enter the enterocytes by passive diffusion, but there is some evidence that carriers may be involved. Inside the cells, the lipids are rapidly esterified,

maintaining a favorable concentration gradient from the lumen into the cells. Unlike the ileal mucosa, the rate of uptake of bile salts by the jejunal mucosa is low, and for the most part the bile salts remain in the intestinal lumen, where they are available for the formation of new micelles. The fate of the fatty acids in enterocytes depends on their size. Fatty acids containing less than 10-12 carbon atoms pass from the mucosal cells directly into the portal blood, where they are transported as free (unesterified) fatty acids. The fatty acids containing more than 10-12 carbon atoms are reesterified to triacylglycerols in the mucosal cells. In addition, some of the absorbed cholesterol is esterified. The triacylglycerols and cholesteryl esters are then coated with a layer of protein, cholesterol, and phospholipid to form chylomicrons. These leave the cell and enter the lymph. In the mucosal cells, most of the triacylglycerol is formed by the acylation of the absorbed 2-monoglycerides, primarily in the smooth endoplasmic reticulum. Some of the triacylglycerol is formed from glycerophosphate. This in turn is a product of glucose catabolism. Cholesterol is readily absorbed from the small intestine if bile, fatty acids, and pancreatic juice are present. Almost all the absorbed cholesterol is incorporated into chylomicrons that enter the circulation via the lymph [159].

1.6.4. Lipid metabolism

Cholesterol and triacylglycerols are the most common dietary lipids and essential for membrane structure, synthesis of steroid hormones, vitamins, bile acids, and energy-storage. Although cholesterol is absorbed from the diet, the body itself can produce sufficient amounts of cholesterol and normally contributes over 50% of daily required cholesterol [160]. Cholesterol is a vital component of the plasma membranes as it determines the fluidity of lipid bilayers. Cholesterol is also essential in the biosynthetis of vitamin D, bile acids, and steroid hormones such as testosterone, estradiol, and cortisol. Triacylglycerols play a central role as energy supply for skeletal and cardiac muscles [160].

Lipids are transported through the body via blood and lymph. To this end the hydrophobic cholesterol (-esters) and triacylglycerols are assembled in lipoprotein particles, which are macromolecular complexes with a hydrophobic core containing the cholesterol esters and triacylglycerols. To render it soluble in an aqueous environment, the lipid core is surrounded by a monolayer of polar phospholipids which also contains

unesterified cholesterol and proteins called apolipoproteins. Apolipoproteins serve as ligands for cell-surface receptors and co-factors/inhibitors of lipases and other enzymes [160].

Table (3): Apolipoproteins associated with lipoproteins

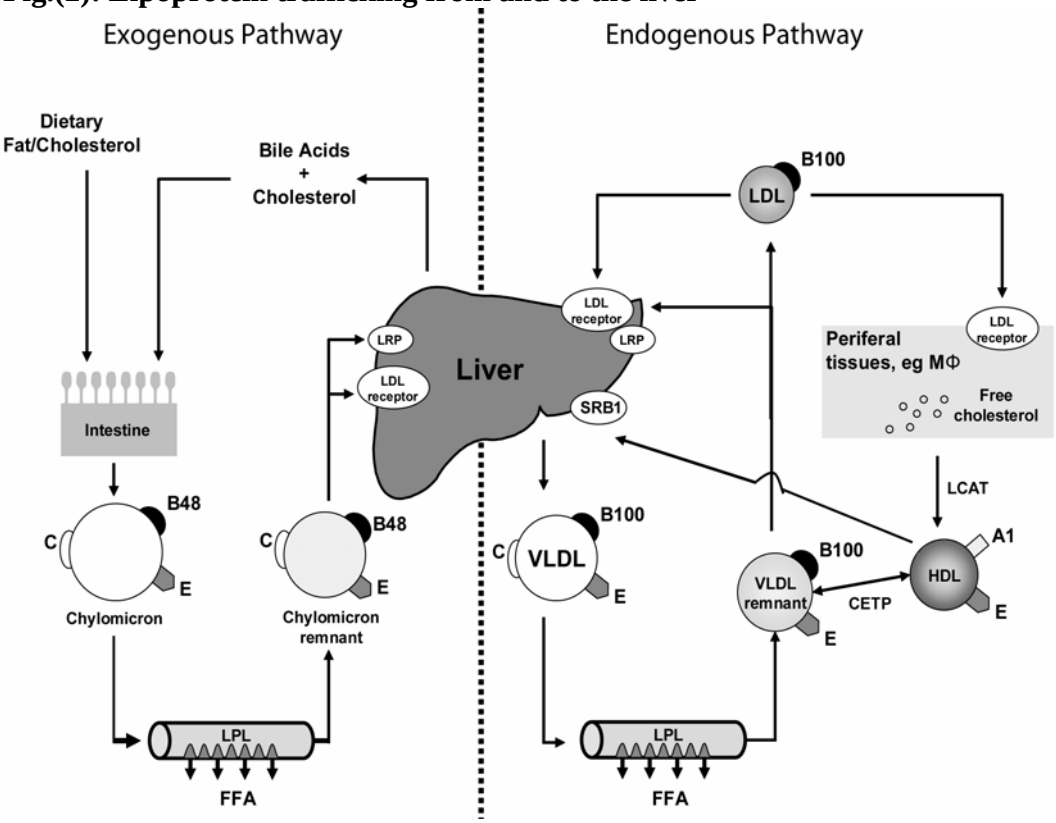
apolipoprotein	**Lipoptrotein association**	**Comments**
apoA1	Chylomicron, HDL	Major protein of HDL, activates LCAT
apoA2	Chylomicron, HDL	Enhances hepatic lipase activity
apoA4	Chylomicron, HDL	Co-factor for LPL activation, may have a role in chylomicron and VLDL secretion and catabolism, activates LCAT
apoA5	HDL	Activates LPL
apoB48	Chylomicron	Exclusively found in chylomicrons, alternative splice product of the apoB100 gene thereby lacking the LDL receptor-binding domain
apoB100	VLDL, IDL, LDL	Ligand for the LDL receptor
apoC1	Chylomicron, VLDL, IDL, HDL	Appears to modulate the interaction of apoE with β-VLDL and inhibit binding of β-VLDL to LRP
apoC2	Chylomicron, VLDL, IDL, HDL	Activates LPL
apoC3	Chylomicron, VLDL, IDL, HDL	Inhibits LPL
apoE	Chylomicron, VLDL, IDL, HDL	Ligand for LDL receptor and LRP

Based on their size, and lipid and apolipoprotein composition, lipoproteins can be categorized into four classes: chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and the high density lipoprotein (HDL). However, heterogeneity exists within each class because of the constant remodeling of their

composition, shape, size, and surface charge, and exchange of protein and lipid constituents [160].

The liver plays a central role in lipid metabolism. Lipoprotein trafficking from and to the liver proceeds along two pathways: the endogenous and the exogenous pathway. Cholesterol transport from peripheral tissues to the liver is often considered as the third pathway [161].

Fig.(2): Lipoprotein trafficking from and to the liver



The exogenous pathway involves the uptake of dietary lipids by the intestine followed by their transport through the body via chylomicrons. After the lipid intake, the triacylglycerols and the cholesteryl esters will be hydrolyzed and reassembled in the intestine to form chylomicrons. These large triacylglycerol -rich chylomicrons containing apoA1, apoA2, apoA4, and apoB48 are secreted into the lymph in order to transport

entrapped lipids to the circulation. In the bloodstream, the chylomicrons exchange apoA1 and associated apoA4 for apoC1, apoC2, apoC3, and apoE with HDL which subsequently results in the formation of the more cholesterol-rich chylomicron remnants due to loss of triacylglycerols to peripheral tissue. ApoC2 is an activator of the enzyme lipoprotein lipase (LPL), which is associated with the capillary endothelium of skeletal muscle, cardiac muscle, and adipose tissue [161]. ApoC2 promoted the interaction of chylomicrons with LPL and allows the hydrolysis of its core triacylglycerols leading to release of free fatty acids which are taken up by surrounding tissue for either oxidation or storage. Triacylglycerols hydrolysis gradually becomes less efficient due to depletion of the LPL co-activator apoC2. Eventually the chylomicron remnants, enriched in cholesteryl esters, apoB48, and apoE are removed from the circulation by the liver mainly via uptake by the apo-receptor which specifically recognizes apoE and apoB100, and by the LDL-receptor related protein (LRP) [162, 163].

The liver produces the triacylglycerols-rich lipoprotein, VLDL, which comprises the endogenous pathway of lipid transport. Some of the VLDL contained triacylglycerols will be derived from internalized chylomicron remnants, but the bulk of the VLDL triacylglycerols are synthesized *de novo* in the liver from fatty acids produced from carbohydrate derived acetyl-CoA [164]. The formation of VLDL starts with the transfer of lipids towards the major structural protein of VLDL, apolipoprotein B100 (apoB100). This transfer is catalyzed by the microsomal triacylglycerols transfer protein (MTP), making this enzyme a key factor in the assembly of VLDL. Subsequently before secretion into the blood circulation, the pre-VLDL particle acquires apoC1, apoC2, apoC3, and ApoE. ApoE plays a critical role when it comes to secretion of VLDL and clearance of VLDL remnants.[165] Similar to chylomicrons, VLDL transports triacylglycerols to skeletal muscle, cardiac muscle, and adipose tissue and also serves as substrate for LPL in the circulation. As a result, VLDL particles gradually reduce in size due to hydrolysis of the triacylglycerols leading to the formation of the more cholesterol-rich IDL. Small sized VLDL and IDL are also referred to as VLDL remnants. These remnants are partially cleared by the liver through an apoE-mediated process while the remainder can be converted to LDL. The conversion to LDL is accompanied not only by

a further loss in triacylglycerols but also by the depletion of apoE and the apoC's. With apoB100 as sole apolipoprotein constituent of LDL, LDL can be cleared by the liver or taken up by peripheral tissue via the LDL receptor. High levels of LDL and/or VLDL remnants are considered important risk factors for atherosclerosis, and are therefore also termed atherogenic lipoproteins [12, 166-168].

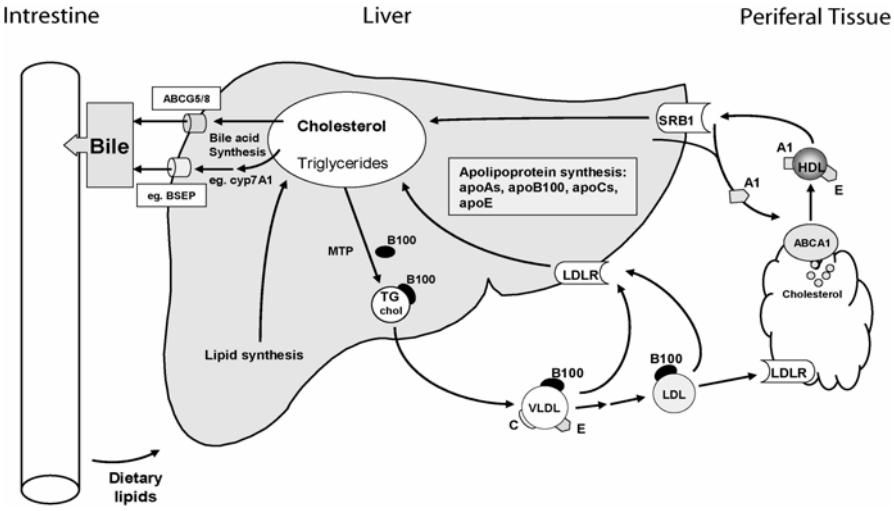
1.6.4.1. Reverse cholesterol transport

While the first two pathways focuses mainly on the transport of triacylglycerols and cholesterol from intestine and liver to peripheral tissue via apoB-containing lipoproteins followed by the clearance of cholesterol-rich lipoprotein remnants, the third pathway involves the transport of cholesterol from peripheral tissue back to the liver where it can be removed from the body via the bile (Fig 1). The reverse transport of cholesterol is mediated by the lipoprotein HDL. HDL is a heterogeneous class of lipoprotein particles existing in different subfractions that differ in apolipoprotein and lipid composition, size, density, and even physiologic function [169, 170]. Generation of HDL starts with the intestinal and hepatic synthesis and secretion of lipid-poor apoA1 containing particles. This ApoA1 'particles' acquires phospholipids and unesterified cholesterol from cell membranes and plasma lipoprotein and turn into discoidal preβ-HDL particle. Nascent pre-HDL particles are potent cholesterol scavengers that take up free cholesterol from peripheral cells, and macrophages. The cholesterol efflux is mediated by the integral membrane protein ATP-binding cassette transporter A-1 (ABCA1), which actively transports cellular cholesterol and phospholipids to the nascent HDL [171, 172]. The importance of ABCA1 in reverse cholesterol was established in patients with Tangier disease who are characterized by HDL deficiency. This disease is caused by a dysfunctional ABCA1 and results in massive accumulation of cholesterol in tissue macrophages with prevalent atherosclerosis [173-175].

Cholesterol taken up by the nascent HDL particles will be esterified by the enzyme lecithin:cholesterol acyltransferase (LCAT), transforming nascent preβ-HDL into larger spherical HDL-particles. Subsequently, the latter HDL particles are subjected to remodeling after interaction with other plasma lipoproteins or tissues. For instance, the

cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesteryl esters for triacylglycerols with apoB-containing lipoproteins. The uptake of HDL cholesterol by the liver is mediated by the scavenger receptor class B1 (SR-B1). Once in the liver, cholesterol can be re-used for lipoprotein assembly, or as a substrate for bile acid synthesis, it can also be secreted directly as free cholesterol into the bile [176].

Fig. (3): Maintenance of lipid homeostasis, is the sum of numerous complex physiological processes both at an organ, and at a cellular level. The organ that plays a key role in the synthesis and catabolism of lipids is the liver (Fig.2). It expresses enzymes that are responsible for synthesis (e.g. fatty acid synthetase and HMG-CoA reductase) of triacylglycerols and cholesterol (esters). Excess of cholesterol can be metabolized in the liver via enzymes such as cholesterol-7-α-hydroxylase and sterol 27-hydroxylase (cyp7A1 and cyp27, respectively), and removed from the body via bile acid synthesis. Further, it is involved in assembly and secretion of the main lipoproteins, i.e. VLDL and HDL, and the elimination of serum lipoproteins via lipoprotein receptors. Most, if not all, apolipoproteins involved in the metabolism of lipoproteins are expressed in the liver. In order to prevent high lipid levels, which correlate with increased risk of cardiovascular disorders, it is essential that the expression of lipid-related genes in the liver is well orchestrated. Several aspects and processes will be highlighted below.



1.6.4.2. Factors that may affect the serum concentration of cholesterol

1. An increase in the amount of cholesterol ingested each day increases the plasma concentration. However, when cholesterol is ingested, this rising level of cholesterol inhibits the enzymes of endogenous cholesterol synthesis, preventing excessive increase

in plasma cholesterol concentration. Because of this feedback control, cholesterol intake does not alter plasma cholesterol concentration significantly [158].

2. A diet high in saturated fat increases serum cholesterol by 15 to 25 % of the normal level. This results from increased acetyl-CoA in the liver which may be used for the production of cholesterol.
3. Lack of insulin or thyroid hormone increases the blood cholesterol concentration. While, excess thyroid hormone decreases the concentration of cholesterol.
4. Ingestion of fat containing highly unsaturated fatty acids usually depresses the blood cholesterol concentration. The mechanism for this effect is not clearly understood, however, this property is presently used as a dietary strategy to lower blood cholesterol level [158].

1.6.5. Plasma lipids and CHD risk

1.6.5.1. LDL cholesterol

The association between plasma total cholesterol (or LDL cholesterol) and CHD risk is well established. Results from a 12-year follow-up of 316 099 men screened for the Multiple Risk Factor Intervention Trial (MRFIT) showed a strong association between serum cholesterol levels and CHD mortality. However, CHD risk and the predictive power of serum cholesterol is significantly modified by the presence of non-lipid risk factors, especially smoking, hypertension and diabetes. For example, a 12-year follow-up of MRFIT subjects showed that the absolute risk of death was at least three times higher for diabetics than for non-diabetics, and that this relationship was amplified by serum cholesterol [177].

1.6.5.2. Relationship between reduced HDL, LDL, elevated triacylglycerols, and cardiovascular disease risk

It is well documented that reduced HDL cholesterol levels are associated with an increased risk of coronary heart disease (CHD). A number of functions of HDL may contribute to this cardioprotective effects, including promotion of cellular cholesterol efflux and direct antioxidant and anti-inflammatory properties. Moreover, low HDL

cholesterol levels are often accompanied by elevated triacylglycerols levels, and this combination has been strongly associated with an increased risk of CHD [125, 178].

Individuals with diabetes mellitus type 2 and CHD tend to have small HDL particles. In addition, hyperinsulinemia and hyper triacylglycerolemia are independently associated with low levels of HDL_2 and small HDL particle size. In individuals with visceral obesity and insulin resistance, small HDL particle size represents another feature of the dyslipidemic profile that is common in these patients. Increased atherogenic potential of small dense LDL appears to be related to a number of physicochemical and metabolic properties of these particles, such properties include reduced LDL receptor affinity, greater propensity for transport into the subendothelial space, increased binding to arterial wall proteoglycans, and susceptibility to oxidative modifications. Although these are *in vitro* findings, they support the concept that small dense LDL contributes to arterial damage in patients with the characteristic dyslipidemia associated with diabetes [179, 180].

1.7. Inflammatory markers

1.7.1. Pathophysiology of inflammatory markers

Serum markers of inflammation provide insight into the pathophysiology of heart diseases and its complications. Testing for C-reactive protein (hs-CRP) which is a nonspecific marker of low-grade systemic inflammation, has received much attention, and several studies have linked elevations of hs-CRP and high risk of coronary diseases. "Distal" indicators of inflammation likewise predict coronary risk, for examples the soluble forms of leukocyte adhesion molecules, (sICAM-1) may reflect elevated levels of proinflammatory cytokines. Interleukin-6 (IL-6) probably provokes the augmented expression of the C-reactive protein (CRP) gene in the liver. Cytokines such as tumor necrosis factor-α (TNF-α) or IL-1 isoforms can in turn stimulate the expression of IL-6 and of the leukocyte adhesion molecules, such as ICAM-1 [181].

Increased levels of cytokines might arise from atheroma themselves. The cytokines might also derive from nonvascular sources and reflect inflammatory states such as chronic infections that may accelerate atherogenesis and its manifestations. Both vascular and

extravascular sources of inflammatory cytokines may prove important to varying degrees in different individuals. Regardless of the source of the inflammatory cytokines, emerging work on serum inflammatory markers supports the notion of a "pathway" of inflammatory activation related to acute coronary events. Prospective epidemiological studies have now shown that measurements of serum inflammatory markers are associated with increased coronary risk [182, 183].

1.7.2. Inflammatory markers levels in overweight and obese adults

Overweight and obese patients may be maintaining a state of low-grade systemic inflammation, increasing their risk for cardiovascular disease. The clue is their consistently above-normal blood concentrations of CRP, a sensitive marker of systemic inflammation [184].

Adipose tissue secretes proinflammatory cytokines and fibrinolytic regulators, such as plasminogen activator inhibitor-1. Among the various cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) seem to play a major role because they are expressed in and released by adipose tissue. These cytokines can influence endothelial function, and induce endothelial expression of chemokines and adhesion molecules, which are central in the early stage of the atherogenetic process [185-187].

Blood markers inflammation, such as C-reactive protein (CRP) predict cardiovascular diseases, suggesting a role for inflammation in the initiation of atherosclerosis, as well as in the precipitation of an acute attack [188].

1.7.3. C-reactive protein

C-reactive protein (CRP) was first described by Tillet and Francis in 1930. It has been a measure of acute phase reactions to inflammation for the last 15 years. Recently improved high sensitive and standardized quantitative assays in serum and cerebrospinal fluid (CSF) have allowed a re-evaluation of its potential as a diagnostic laboratory test. CRP is a serum glycoprotein produced by the liver during acute inflammation. Because it disappears rapidly when inflammation subsides, its detection signifies acute

inflammatory process. Further, by serial measurements important information can be obtained on the resolution or continuation of the inflammatory process. The function of CRP is thought to be related to its role in the innate immune system. Similar to immunoglobulin IgG, it activates complement, binds to Fc receptors and acts as an opsonin for various pathogens. Interaction of CRP with Fc receptors leads to the generation of proinflammatory cytokines that enhance inflammatory response [189].

1.7.3.1. CRP as predictor of cardiovascular events

Recent reports indicate that inflammation may be associated with atherosclerosis, and high levels of CRP may indicate atherosclerosis. Myocardial infraction frequently occurs at the end of a long process of inflammation-mediated atherosclerosis. Measurement of markers of inflammation has been proposed as a method to predict CVD risk. CRP may be a useful marker of subclinical atherosclerosis and cardiovascular risk. CRP has been positively linked to cardiovascular diseases. CRP induces adhesion molecule expression in human endothelial cells supporting the hypothesis that CRP play a direct role in promoting the inflammatory component of atherosclerosis. Thus CRP presents a potential target for the treatment of atherosclerosis [190].

1.7.4. Interlukin-6 (IL-6)

Interlukin-6 is a cytokine secreted by immune cells during inflammatory conditions, but is also released by adipose tissue and by contracting skeletal muscle in the absence of inflammation. Although little is known about the role of this release from muscles and adipose tissue, it is possible that IL-6 is a key modulator of lipid homeostasis and metabolism. Interleukin-6 expression and circulating levels correlate directly with obesity, and weight loss lowers circulating levels. In addition elevated IL-6 in plasma is a predictor of the development of cardiovascular disease and diabetes [191, 192].

1.8. Objectives

1.8.1. General objectives

This study was designed to examine the associations of several adiposity measures including total adiposity (BMI and fat mass) and body fat distribution (waist-to-thigh ratio, waist circumference) and total fat mass, with lipid profile parameters in women Also to find out these measures of obesity in relation to different inflammatory markers such as the CRP and IL-6.

1.8.2. Specific objectives

The study is designed to:

- Measure the plasma concentration of lipid profile parameters (including: total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol.) in both non obese and obese subjects.
- Measure the concentration of inflammatory markers (CRP and IL-6) in both non obese and obese subjects.
- Correlate the fat distribution and fat composition with the concentration of the inflammatory markers.

CHAPTER TWO

Materials and Methods

This was a descriptive study carried out in Khartoum state. Two hundred twenty four female medical students were enrolled in this study after having their consent. Anthropometrical measurements and biochemical analysis were performed for all participants and they were divided into two groups: non obese and obese according to their BMI (kg/m^2).

2.1. Subjects:

Individuals with BMI $\geq$30 kg/m^2 and apparently healthy were enrolled as obese in this study. Those who were pregnant, diabetic or receiving medications were excluded from the study. Non obese criteria: clinically and physically fit, non pregnant females of BMI between 18.5 -24.9 kg/m^2, were enrolled in the study as control subjects.

2.2. Blood samples

Subjects were fasting for 10-12 hours and they refrain from high impact physical activity the preceding day. Five ml of blood was collected at 09:00 a.m. from antecubital vein into EDTA vacuotainer, plasma was obtained by centrifugation and stored at -20^oC for biochemical analyses.

2.3. Measurements and investigations

2.3.1. Blood pressure:

Systolic and diastolic blood pressures were measured using a calibrated mercury sphygmomanometer after the subject had been seated for 30 min. The average of two measurements taken on the right arm was recorded. Subjects were considered hypertensive when their systolic blood pressure (SBP) was $\geq$130 mmHg, and their diastolic blood pressure (DBP) was $\geq$85 mmHg [194, 195].

2.3.2. Anthropometric measurements

2.3.2.1. Body mass index

Body weight was measured on a standard balance beam scale to the nearest 0.1 kg. Height was measured barefoot; to the nearest 0.1 cm by using a wall-mounted stadiometer. BMI was calculated as weight (kg)/height in ($meter^2$) [196].

2.3.2.2. Waist and thigh circumferences

Waist and thigh circumferences were measured with a plastic tape meter which measures to the nearest 0.1 cm. Waist circumference was taken at the largest abdominal circumference, and thigh circumference was measured midway between the inguinal crease and the proximal border of the patella [196].

2.3.2.3. Waist-hip ratio

WHR were calculated from the duplicate measurement of the minimal waist circumference to the circumference of the maximal gluteal protuberance. The hip circumference was taken at the largest standing horizontal circumference of the buttocks [197].

2.3.2.4. Skinfold thickness

The recommendations of the committee on nutritional anthropometry of the Food and Nutrition Board of the National Research Councils, were followed [198]. A Holtain caliper was used with a constant caliper pressure of 102 g/mm^2. The caliper had rectangular jaw faces measuring 40 mm. Three to five measurements were taken on the right side of the body with subjects standing and the mean was expressed as skin-fold score. The skin-fold thickness was measured to the nearest mm. Except for low values (usually less than 5 mm or less), in such case it will be taken to the nearest 0.5 mm. Four sites were chosen: biceps, triceps, sub-scapular and suprailiac. Body density was calculated in g/cc derived from the sum of these four skin-fold thickness scores using the equations of Durnin and Womersley [199].

Percentage body fat content was calculated from the body density values using the Durnin and Womersley equation [200]. The precision of this method in estimating percentage body fat content is reported to be ± 3.5% body fat.

2.3.2.4.1.1. Triceps skinfold thickness measurement

The volunteers were asked to stand with her feet together, shoulders relaxed, and arms hanging freely at the sides. Standing on the volunteer's right side, location of a point on the posterior surface of the right upper arm is made at mid way on a line joining the tip of the posterior border of the acromion process superiorly, and the tip of the olecranon

process inferiorly. The point was marked with a pen. The fold of skin and subcutaneous tissue was grasped gently with the thumb and fore fingers, approximately 1.0 cm above the point at which the skin was marked, with the skinfold parallel to the long axis of the upper arm. The jaws of the caliper were placed perpendicularly to the skinfold for 3 seconds. Holding the caliper, measurement of the fold was recorded to the nearest 1 mm with the fingers continue to hold the fold [200].

2.3.2.4.1.2. Biceps skinfold thickness measurement

The same steps of the triceps skinfold measurement was followed but with the measurement done from the front of the arm. The arm should be rotated so that the palm is facing interiorly. Location of the point of the mid arm level was made and grasping the skinfold with the thumb and forefinger. The placement of the caliper and measurement was done with the fingers continue to hold the fold [200].

2.3.2.4.1.3. Subscapular skinfold measurement

The back of the individual is exposed, and the inferior angle of the right scapula was palpated. The skinfold directly below (1.0cm) and medial to the inferior angle is grasped. The skinfold forms a line about 45^{O} with a horizontal line below extending to the elbow. The jaws of the caliper were placed perpendicular to the length of the fold. Measurement of the skinfold was done with the fingers continue to hold the fold [200].

2.3.2.4.1.4. Suprailiac skinfold measurement

With the volunteer in erect position, the arms were slightly abducted to help access to the site. The suprailiac skinfold is measured in the mid axillary line immediately superior to the iliac crest. The fold so grasped at an oblique angle just posterior to the mid axillary line, below the natural cleavage line of the skin. Placement of the caliper and recording was done with the fingers holding the fold [200].

2.3.2.4.2. Estimation of body fat

The logarithm of the sum of the four skinfolds was calculated. The body density was then calculated using each of the following equations according to the age and gender of the individual.

Table (4) Determination of body density

Age rang	Equation for men	Age rang	Equation for women
17-19	D = 1.1620-0.053x(10g∑)	17-19	D = 1.1549-0.0678x(10g∑)
20-29	D = 1.1631-0.0532x(10g∑)	20-29	D = 1.1599-0.0717x(10g∑)
30-39	D = 1.1422-0.0455x(10g∑)	30-39	D = 1.423-0.0532x(10g∑)
40-49	D = 1.1620-0.0700x(10g∑)	40-49	D = 1.333-0.512x(10g∑)
50+	D = 1.1715-0.0779x(10g∑)	50+	D = 1.1339-0.0545x(10g∑)

D = Density (10g∑) = Logarithm of the sum of the 4 skinfolds thickness fat mass is calculated using the following formula

Fat mass (kg) = **[**body weight (kg) X (4.5/D) – 4.5**]** [200].

Where D was obtained from the equations shown in the table above.

The percentage of body fat was then calculated using the body weight and the fat mass.

% body fat = (fat mass/body weight)X100

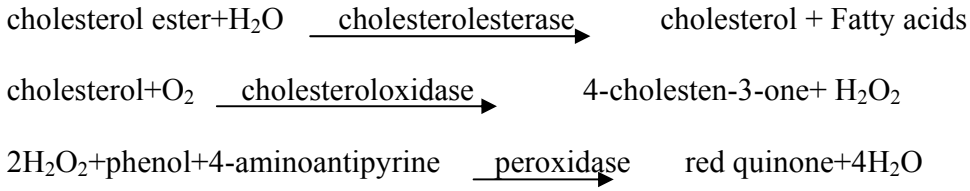
2.3.3. Biochemical analysis

2.3.3.1. Lipid profile parameters

2.3.3.1.1. Measurement of total cholesterol

Total Cholesterol was measured by **e**nzymatic colorimetric method (Diagnosticum Rt, Hangary).

The cholesterol esters of the sample are hydrolysed by cholesterol esterhydrolase (ChEH). 4-Cholesten-3-one and H_2O_2 are then formed from the released free cholesterol by cholesterol oxidase (ChOD). A measurable red quinonimine derivative which absorb light at 505 nm is formed from hydrogenperoxide (H_2O_2) and 4-aminoantipyrine in the presence of phenol and peroxidase (POD) [201].



The reference value for serum cholesterol was: 2.8-5.2 mmol/l (1.09-2.02 g/l)

Reagents

1. Reagent (R1)

pipes buffer,pH=……..……6.90 50 mmol/l

phenol……. ……24 mmol/l

sodium cholate ……..0.5 mmol/l

4-aminoantipyrine ……..0.5 mmol/l

cholesterol esterase …….180 U/l

sholesterol oxidase …….200 U/l

peroxidase …….1000 U/l

2. Reagent (R2)

cholesterol standard …….……..5.17 mmol/l (2 g/l

Stored at 2-8°C and protected from light reagents are stable:

unopened: up to the expiry date stated on the label, after opening, they are stable for 30 days.

Assay conditions:

Wavelength:…… 505 (480-520) nm

Temperature: ……..37°C

Cuvette: ……1 cm light path

Read against: …….....reagent blank

Method: ……..endpoint (increasing)

Pipette into cuvette:

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and read the absorbance (A) after 5 minutes incubation.

Calculation

(A sample/ A standard) X C standard = C sample

A = Absorbance, C = Concentration

Quality control: The analysis of control material in both the normal and abnormal ranges with each assay was recommended for monitoring the performance of the procedure.

2.3.3.1.2. Measurement of triacylglycerol

Triacylglycerols was measured by enzymatic method using: Triacylglycerols OSR6x33, OLYMPUS kit.

This is an enzymatic colour test for quantitative determination of triacylglycerol in serum and plasma. This procedure is based on a series of coupled enzymatic reactions. The triacylglycerols in the sample are hydrolysed by a lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3- phosphate. The glycerol-3-phosphate is oxidised by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The H_2O_2 is used to oxidatively couple *p*-chlorophenol and 4-aminoantipyrine (4-AAP) catalysed by peroxidase (POD) to give a red dye with an absorbance maximum at 500 nm. The increase in absorbance at 520/600 nm is proportional to the triacylglycerol content of the sample [202].

triacylglycerol + $3H_2O$ $\xrightarrow{\text{Lipase}}$ glycerol + 3 fatty Acid

glycerol + ATP $\xrightarrow{\text{GK, } Mg^{2+}}$ glycerol-3-phosphate + ADP

glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-AAP + 4-chlorophenol $\xrightarrow{\text{POD}}$ quinoneimine (Red) + HCL + $2H_2O$

Reference Range

Normal: …… .<1.70 mmol/L High:…… 2.26 - 5.64 mmol/L
Borderline high:…… 1.70 - 2.25 mmol/L Very high:……..≥ 5.65 mmol/L

2.3.3.1.3. Measurement of HDL cholesterol

HDL Cholesterol was measured by enzymatic method (Stanbio Laboratory, Boeme, Texa)

Principle: Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol fractions are precipitated from serum or plasma by means of magnesium chloride dextran sulfate reagent. According to Finley *et al*. High density lipoprotein (HDL) cholesterol is then determined in the supernatant fluid using a cholesterol reagent and the derived dilution factor in the calculation [203].

Reagents

HDL cholesterol precipitin reagent.

Reagent contains the following active ingredients at stated concentrations.

Magnesium chloride	119.5 mmol/L
Dextran sulfate (500.000 M.W.)	1.1 % W/V

HDL cholesterol standard.

Buffered aqueous solution of.

HDL Separation procedure

To 0.5mL plasma in a test tube add 0.05 HDL precipitating reagent.

Mix well and allow to stand 5 minutes

Centrifuge for 10 minutes at high speed (1000 x g).

Use clear supernatant, which contain HDL cholesterol as sample

Automated Analyzers

Parameters:

Wavelength……..……...…..500nm

Reaction type…….....Endpoint

Reaction direction……..……..Increasing

Reaction temperature……..…...…….37^{O}C

Sample/reagent ratio……..1:40

Equilibration time……...…..3 Seconds

Read time…….……...4 Seconds

Lag time…….……300 Seconds

Blank absorbance limit……..0.30 A

High absorbance…….…….…….....2.000A

Standard……..……...55* mg/dL

Low normal……...….....30 mg/dL

High normal…….……..……..…….85 mg/dL

Linearity……..125 mg/dL

Above parameters should employed in programming automated analyzers for HDL cholesterol.

Test performance

1. Pipet into cuvets the following volumes (ml) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Reagent	1.0	1.0	1.0
Standard	-	0.05	-
Sample (supernatant)	-	-	0.025

2. Incubate all cuvets a 37 ^{O}C for 5 minuets or at room temperature for 10 minutes.

3. Read and U vs RB at 500 nm within 60 minutes.

Results

Values are derived by the following equation

HDL Cholesterol (mg/dL) = (Ahdl/As)* 55#

Where Ahdl and As are the absorbance values of unknown HDL fraction and standard, respectively, and 55 the concentration of the standard (mg/dL) multiplied by the dilution factor of 1.1.

HDL Cholesterol Standard, 5O mg/dL x 1.1 = 55 mg/dL

NOTE: Samples having cholesterol values greater than 750 mg/dL arc diluted 3-fold (1+2) with normal saline (sodium chloride, 8.5 g/L). the assay repeated and results multiplied by the dilution factor of 3.

Expected Values

The expected values for serum HDL cholesterol are as follows:

Males: 30 - 70 mg/dL Females: 30-85 mg/dL

2.3.3.1.4. Measurement of LDL cholesterol

According to Friedewald *et al*. LDL can be calculated as follows:

LDL = Total cholesterol – HDL – TAG/5 [204]

Normal range 70-170mg/dl

2.3.3.2. Inflammatory markers

Cytokines were measured by using Enzyme Link Immuno-Sorbent Assay (ELISA).

2.3.3.2.1. Measurement of C-reactive protein

Enzyme immunoassays for the quantitative high sensitive determination of C-reactive protein in human serum and plasma. (hsCRP ELISA - DiaMed EuroGen Ref. No. 740011, Belgium)

Principle of the CRP ELISA

Microtiter plates coated with anti-CRP antibody are incubated with diluted standard sera and patient samples. During this incubation step, CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies. After removal of the unbound conjugate, the plates are incubated with a chromogen solution containing tetramethylbenzidin and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells. The enzymatic reaction is stopped by the addition of 2N H_2SO_4 and the absorbance at 450 nm are determined. A standard curve is obtained by plotting the absorbance versus the corresponding standard values. The concentration of CRP in patient samples is determined by interpolation from the standard curve [205].

Reagent

1. Coated Microtiterstrips - 12 x 8-well strips coated with monoclonal antibodies to human CRP.
2. Standard Sera - 5 vials, each containing 1/10 prediluted CRP standard solutions (0.2 ml): 0 - 0.4 - 1 - 5 - 10 µg/ml. Calibrated against the NIBSC 1st International standard, 85/506. Contain 0,09 % NaN_3 and antimicrobial agents as preservatives.
3. Conjugate - 1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 ml). Contains antimicrobial agents and an inert red dye.
4. Specimen dilution buffer - 2 vials, containing 100 ml dilution buffer at working strength. Contain 0.09 % NaN_3 as preservative and an inert green dye.
5. Washing solution - 2 vials containing 25 ml 20X concentrated phosphate buffered washing solution. Contain antimicrobial agents.
6. Chromogen Solution: 1 vial, containing 25 ml of a solution containing H_2O_2 and tetramethylbenzidin.
7. Stopping Solution - 1 vial, containing 10 ml of 2N H_2SO_4

Assay procedure

1. The 10X prediluted standard sera (2) are diluted 1:100 as follows: pipette 10 µl of each calibrator into separate tubes. Add 990 µl of specimen dilution buffer (4) and mix carefully.
2. The patient samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient sample into separate dilution tubes and add 990 µl of specimen dilution buffer (4). Mix thoroughly. Add 450 µl of specimen dilution buffer to 50 µl of these 100 x prediluted samples. Mix thoroughly. The dilutions should be used within 8 hours.
3. Pipette 100 µl of the diluted calibrators and samples into adjacent wells of the microtiterplate (1). It is suggested to pipette the calibrators in duplicate.
4. Incubate the covered microtiterstrips for 30 ± 2 min at room temperature.
5. Wash the microtiterstrips three times with washing solution. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the plates for 2-3 min. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted plates on adsorbent paper.
6. Add 100 µl of conjugate solution (3) and incubate the covered microtiterstrips for 30 ± 2 min at room temperature.
7. Repeat the washing cycle as described in 5.
8. Add 100 µl of Chromogen (6) Solution to each well.
9. Incubate for 10±2 min at room temperature. Avoid light exposure during this step.
10. Add 50 µl of Stopping Solution (7) to each well.
11. Determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

    Results: The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. log/linear) is constructed. Use the average absorbance of each patient sample obtained in the hsCRP ELISA to determine the corresponding value by simple interpolation from the curve.

2.3.3.2.2. Measurement of interleukin-6 (IL-6)

Interleukin-6 was measured by a solid-phase, enzyme labeled assay using IMMULITE and IMMULITE 1000 analyzers — for *in vitro* quantitative measurement of interleukin 6 (IL-6) in serum or EDTA plasma (Cat. No.LK6P1)

Principle: IMMULITE/IMMULITE 1000 IL-6 is a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay [206].
Incubation cycles: 2 x 30 minutes.

Volume required: 100 uL plasma. (Sample cup must contain at least 250 uL more than the total volume required).

Storage: 1 day at 2-8°C or 6 months at -20°C.

Reagents: Store at 2-8°C. dispose of in accordance with applicable laws.

Materials supplied: Components are a matched set. The barcode labels are needed for the assay.

IL-6 test units (L6P1)

Each barcode-labeled unit contains one bead coated with a monoclonal murine anti-IL-6 antibody. Stable at 2-8°C until expiration date.

LK6PZ: 50 units. **LK6P1:** 100 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

IL-6 Reagent Wedges (L6PA, L6PB)

With barcodes. **L6PA;** 7.5 mL of a protein/buffer matrix, with preservative.L6PB: 7.5 mL alkaline phosphatase

(bovine calf intestine) conjugated to polyclonal sheep anti-IL-6 antibody in buffer, with preservative. Store capped and refrigerated: stable at 2-8"C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.

LK6PZ: 1 set. **LK6P1**: 2 sets.

IL-6 Adjusters (L6PL, L6PH)

Two vials (low and high) of lyophilized IL-6 in a protein/buffer matrix, with preservative. Reconstitute each vial with 3.0 mL distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved.

Stable at 2-8°C for 3 days after reconstitution, or for 6 months (aliquotted) at -20°C.

LK6PZ; 1 set. **LK6P1**:1 set.

Kit components supplied separately

IL-6 sample diluent (L6PZ) For the manual dilution of patient samples.

One vial containing 25 mL of lL-6-free protein/buffer matrix, with preservative.

Stable at 2-8°C for 14 days after opening,or for 6 months (aliquotted) at -20°C.

LSUBX: chemiluminescent substrate

LPWS2: probe wash module

LKPM: probe cleaning Kit

LCHx-y: sample cup holders (barcoded)

LSCP: sample cups (disposable)

LSCC: sample cup caps (optional)

LILCM: A bi-level, human serum-based IMMULITE cytokine control module containing IL-6.

Normal serum IL-6 levels < 3 pg/ml

2.3.3.3. Blood glucose

Fasting blood glucose was measured using the hexokinase method (Advia 1650 Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany).

Principle of the method: Glucose present in the plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured at 515mm. Blood glucose values are presented as normal (≤110 mg/dL) or high (>110mg/dL) [207, 208].

CHAPTER THREE

Results

3.1. Participants characteristics

Two hundred twenty four female medical students who met the selection criteria were enrolled in the study. There was no difference between the study groups in age. The mean ± SD of age of non obese and obese was 19.65 ± 1.18 and 20.33 ± 1.86 05 respectively (p >0.05). (Table 5)

3.2. Anthropometric measurements

The mean ± SD of the weight for non obese and obese females was found to be 68.55± 1.86 and 93.46 ± 8.96 respectively. This difference was statistically significant with *P value* < 0.000. (Table 5)

The mean ± SD of the height for non obese and obese females was found to be 1.62 ± 0.06 and 1.60 ± 0.06 meter respectively. There was no statistical significant difference (Table 5). No significant difference was observed between Log_{10} weight and log_{10} predicted weight for height in non obese subject. On the other hand significant difference was found in Log_{10} weight and log_{10} predicted weight for height in obese subjects (Table 6). BMI obtained by the formula, weight (kg)/height (m^2) was found to be statistically higher in obese compared to the non obese females (Table 5). 39.1% of the participants with BMI of ≥30 kg/m2 (Fig. 4). In non obese and obese, BMI was significantly correlated to %BF, waist circumference, thigh circumference, biceps, suprailic, and triceps (Figures 5, 6, 7, 8, 9 and 10).

Waist circumference for non obese and obese females had a mean ± SEM of 72.85 ± 0.69and 98.49 ± 1.55mm respectively This difference was statistically significant with *P value* < 0.000 (Table 5). Only 26.7% of the participant had WC of >88Cm (Fig. 1).
Thigh circumference for non obese and obese females has a mean ± SEM of 50.87 ± 0.50 and 66.48 ± 0.86 mm respectively This difference was statistically significant with *P value* < 0.000 (Table 5).

Hip circumference for non obese and obese females has a mean ± SEM of 100.73 ± 0.62 and 124.77 ±1.27 mm respectively This difference was statistically significant with *P*

value < 0.000. (Table 5). Figure 11 shows waist, wrist, thigh and hip circumference of non obese and obese females.

Mean ± SEM of the waist / hip ratio for non obese and obese females was found to be 0.72 ± 0.05 and 0.78 ± 0.06 respectively. Only 5.4% of all participants in the study had WHR of >0.85(Fig. 12), while 14.5% of obese subject with WHR >0.85 (Fig. 13)

Skinfold thickness biceps for non obese females was 8.92 ± 3.5376 while for obese females was found to be 17.605 ± 5.2502 this difference was statistically significant with *P value* 0.000. Skinfold thickness triceps for non obese females was 18.257 ± 0.42 while for obese females was found to be 29.923 ±0.79 this difference was statistically significant with *P value* 0.000. Skinfold thickness subscapular for non obese females was 17.39 ± 0.42 mm while for obese females was found to be 30.42 ± 1.36 mm, this difference was statistically significant with *P value* 0.000. Skinfold thickness suprailiac for non obese females was 23.174 ± 0.41 mm while for obese females was found to be 30.383 ± 1.02 mm, this difference was statistically significant with *P value* 0.000. The body fat percent which is calculated using the above skinfold thickness readings for non obese and obese females was found to be 30.17 ± 3.32 and 37.14 ± 3.92 (mean ± SEM) respectively. This difference was statistically significant with *P value* <0.000.(Table 5)

Blood pressure was measured for all subjects. In non obese subjects, it has mean ± SEM of 116 ± 0.99 systolic and 74.5 ± 0.77 diastolic, while in obese it was found to be 122 ± 1.18 systolic and 80.1 ± 0.89 diastolic. This difference was statistically significant with *P value* <0.001(Table 5). Figure (14) shows that only 21.8% of obese females has SBP >130, and 32.8% of them has DBP >85mmHg.

3.3. Biochemical analysis

3.3.1. Lipids profile

The mean ± SEM cholesterol, LDL, HDL, and TAG for non obese subjects was 136.0 ± 3.23, 76.95 ± 3.43, 44.0 ± 1.20 and 75.51 ± 3.71 mg/dl respectively while for obese was found to be 148.2 ± 3.03, 92.96 ± 3.33, 37.42 ± 1.36 and 90.79 ± 4.41.0 mg/dl respectively .There was statistically significant difference between the two groups ($P < 0.05$) (Table 7).

As shown in table 3 the mean ± SEM, fasting blood glucose for non obese and obese was found to be 102 ± 0.655 and 106.19 ± 1.82 mg/dl respectively. This difference was not significant, (*P value* 0.101), however, the means of the two groups are within the normal range of fasting blood sugar

3.3.2. Inflammatory marker

The mean ± SEM of CRP for non obese and obese females was found to be 0.305 ± 0.09 and 6.256 ± 0.78 respectively this difference was statistically significant with *P value* < 0.008 (Table 7)

The mean ± SEM of IL-6 for non obese and obese females was found to be 5.76 ± 0.34 and 5.16 ± 0.73 respectively. This difference was not significant, *P value* 0.101. (Table 7)

Correlation between BMI and waist, thigh and hip in obese females was shown in table 8 and it was statistically significant *P value* 0.000. BMI was found to be inversely correlated with HDL. *P value* <0.01 (Table 9).

WHR in obese females was positively correlated with BMI (*P*<0.01) (Table 10), the data presented in table 11 indicate a positive correlation of WHR with % fat mass (r=0.276, *P* <0.02).

Figures 15 show a positive correlation of WC with body fat mass in non obese and obese females.

CRP levels of the obese females correlated positively with %BF (*r*=0.290, *P* <0.05), BMI (r=0.504, *P* <0.01), hip circumference (*r*=366, *P* <0.0130), (Tables 12 & 13). CRP was high (>3 mg/l) in 13 of the studied obese females (Table 14 & Fig. 16). Higher percentage of obese females showed normal blood pressure, normal fasting blood sugar, normal total cholesterol and normal CRP (Fig. 17 & Table 14).

Table (5): Demographic and anthropometric characteristics of all participants (mean ± SEM).

	Non obese (n = 141)	Obese (n =64)	*p* value
Age, y	19.65 ± 1.18	20.33 ± 1.86	
BW, kg	68.55 ± 1.86	93.46 ± 8.96	0.000
Height, m	1.62 ± 0.06	1.60 ± 0.06	
BMI, kg/m^2	20.6 ± 0.21	36.1 ± 0.57	0.000
Waist, cm	72.85 ± 0.68	98.49 ± 1.55	0.000
Thigh, cm	50.87 ± 0.50	66.48 ± 0.86	0.000
Hip, cm	100.47 ± 0.62	124.77 ± 1.27	0.000
WHR	0.72 ± 0.05	0.78 ± 0.06	
Biceps, mm	8.92 ± 0.23	17.6 ± 0.65	0.000
Triceps, mm	18.25 ± 0.43	29.92 ± 0.79	0.000
S-Scapular, mm	17.39 ± 0.42	30.42 ± 1.37	0.000
Suprailiac, mm	23.17 ± 0.41	30.38 ± 1.03	0.000
%Body fat	30.17 ± 3.32	37.14 ± 3.92	0.000
BPS, mmHg	116 ± 0.99	122 ± 1.18	0.001
BPD, mmHg	74.5 ± 0.77	80.1 ± 0.89	0.000

P value significant at the 0.05 level.

Table (6): Log_{10} weight and log_{10} predicted weight for height in non obese and obese subjects

	Log_{10} Weight	Log_{10} Predicted Weight	*P* value
Non obese	1.726 ± 0.047	1.726 ± 0.045	
Obese	1.9410 ± 0.06	1.670 ± 0.04 **	0.000

P value significant at the 0.05 level.

Table (7): Lipid profile, fasting blood glucose, CRP and IL-6 values of non obese and obese females (mean ± SEM).

	Non obese (n = 102)	Obese (n = 63)	*p* value
T. Choles., mg/dl	136.0 ± 3.23	148.2 ± 3.03	0.007
LDL, mg/dl*	76.95 ± 3.43	92.96 ±3.33	0.007
HDL, mg/dl	44.0 ± 1.20	37.43 ± 1.36	.0000
TAG, mg/dl	75.51 ± 3.71	90.79 ± 4.41	0.009
CRP mg/l	0.305 ± 0.09*	6.256 ± 0.78*	0.008
IL-6 pg/ml	5.76 ± 0.34	5.16 ± 0.73	0.101
Fasting Glucos, mg/dl	96.1 ± 12.95	102.19 ±13.1	0.101

P value significant at the 0.05 level.

Table (8): Correlations of BMI in obese with waist, wrist, thigh and hip.

	Waist	Wrist	Thigh	Hip
r-Pearson	0.886**	0.562**	0.869**	0.915**
p	0.000	0.000	0.000	0.000

** Correlation significant at the 0.01 level.

Table (9): Correlations of BMI in obese with total cholesterol, LDL, HDL, and TAG.

	T Choles.	LDL	HDL	TAG
r-Pearson	0.083	0.151	-.293**	0.058
p	0.398	0.131	.002	0.554

** correlation significant at the 0.01level.

Table (10): Correlations of WHR in obese with wrist, biceps, triceps, and suprailic.

	Wrist	Biceps	Triceps	Suprailic
r-Pearson	0.338**	0.376**	0.189	0.184
p	0.006	0.002	0.132	0.143

** correlation significant at the 0.01level.

Table (11): Correlations of WHR ratio in obese with BMI and %fat mass.

	BMI	%Fat mass
r-Pearson	0.340**	0.276*
p	0.000	0.02

** Correlation significant at the 0.01 level.
* Correlation significant at the 0.05 level.

Table (12): Correlations of CRP in obese with waist, wrist, thigh and hip.

	Waist	Wrist	Thigh	Hip
r-Pearson	0.307*	0.218	0.326*	.366*
p	0.04	0.151	0.029	0.013

* correlation significant at the 0.05 level.

Table (13): Correlations of CRP in obese with BMI and % fat mass.

	BMI	%Fat mass
r-Pearson	0.504**	0.290*
p	0.000	0.05

** Correlation significant at the 0.01 level.
* Correlation significant at the 0.05 level.

Table.(14): Anthropometric, biochemical characteristics and blood pressure in obese females with CRP <3 mg/l and obese females with CRP of ≥3mg/l.

	Obese CRP <3 mg/l (n =47)	**Obese CRP ≥3 mg/l (n =13)**	***p* value**
BMI, kg/m^2	35.20 ± 4.56	37.4 ± 4.42	……
Thigh, cm	62.45 ± 5.4	68.3 ± 8.3	0.05
Hip, cm	117.3 ± 8.5	128.7 ± 11.5	0.01
WHR	0.70 ± 1.3	0.80 ± 1.1	…….
BPS, mmHg	113.7 ± 10.1	123.5 ± 8.4	0.01
BPD, mmHg	74.5 ± 7.9	81.6 ± 5.7	0.01
T. Choles., mg/dl	138.5 ± 25.9	153.9 ± 20.6	0.000
LDL, mg/dl*	86.6 ± 29.0	98.1 ± 21.6	……..
HDL, mg/dl	35.5 ± 11.2	37.5 ± 10.4	……
TAG, mg/dl	87.2 ± 36.4	91.5 ± 33.7	……
CRP, mg/l	0.93 ± 0.9	9.85 ± 3.9	0.000
IL-6, pg/ml	3.9 ± 2.1	4.3 ± 2.9	……
Fasting glucos, mg/dl	91.4 ± 14.4	97.8 ± 10.1	…….

Fig. 4: Classification of study participants as obese using BMI>30, %BF >30, and WC >88 Cm.

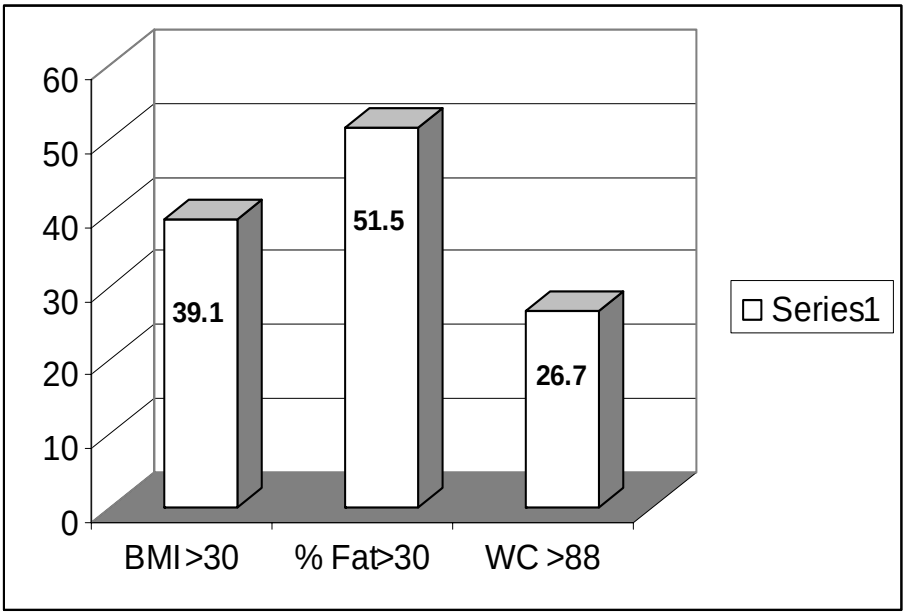


Fig. 5: Correlation between BMI and % body fat in non obese and obese females.

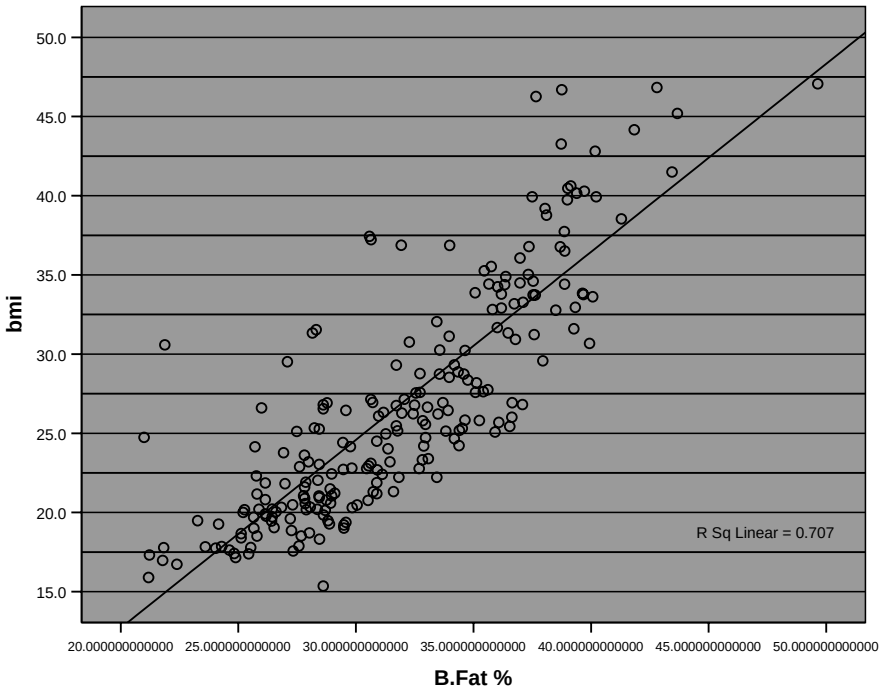


Fig.6: Correlation between waist circumference and BMI in non obese and obese females.

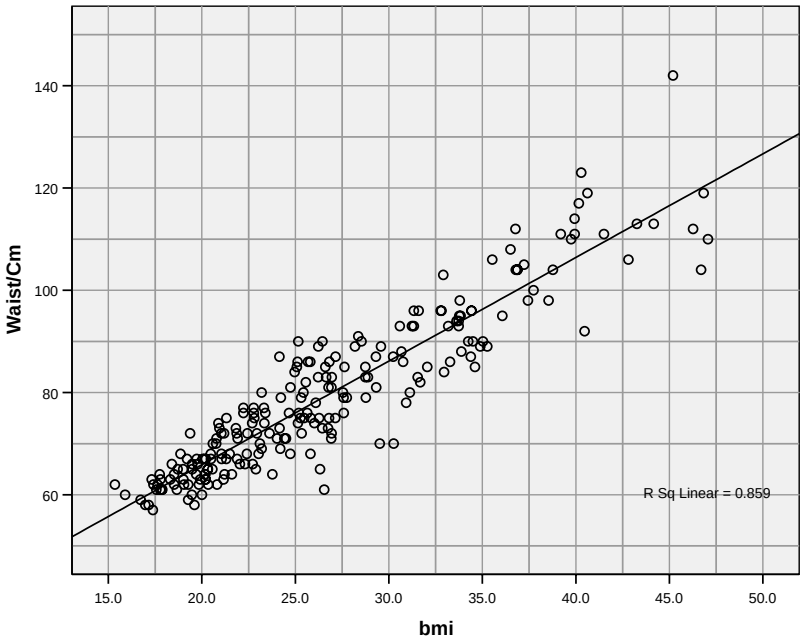


Fig.7: Correlation between BMI and thigh circumference in non obese and obese females .

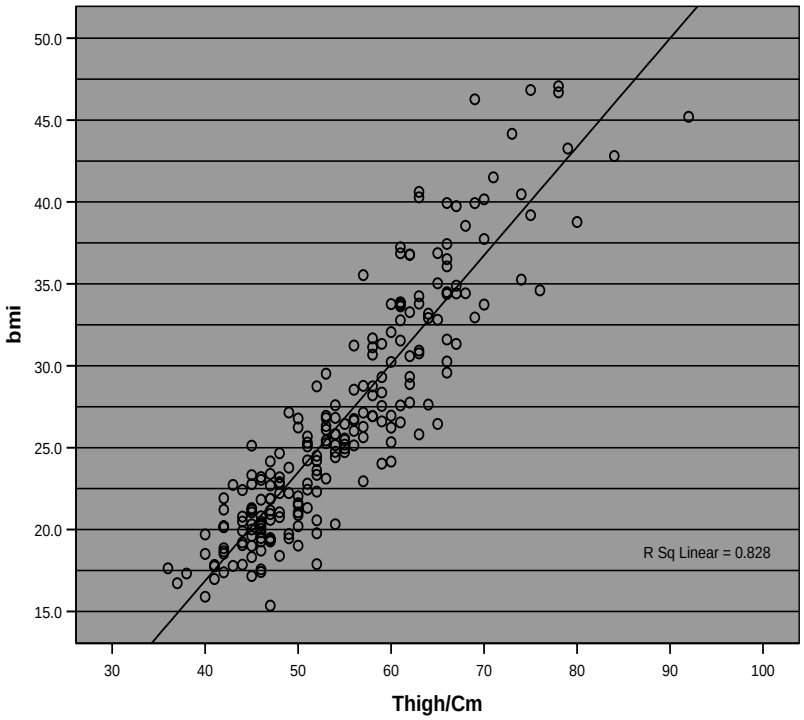


Fig.8: Correlation between BMI and biceps skinfold thickness in non obese and obese females.

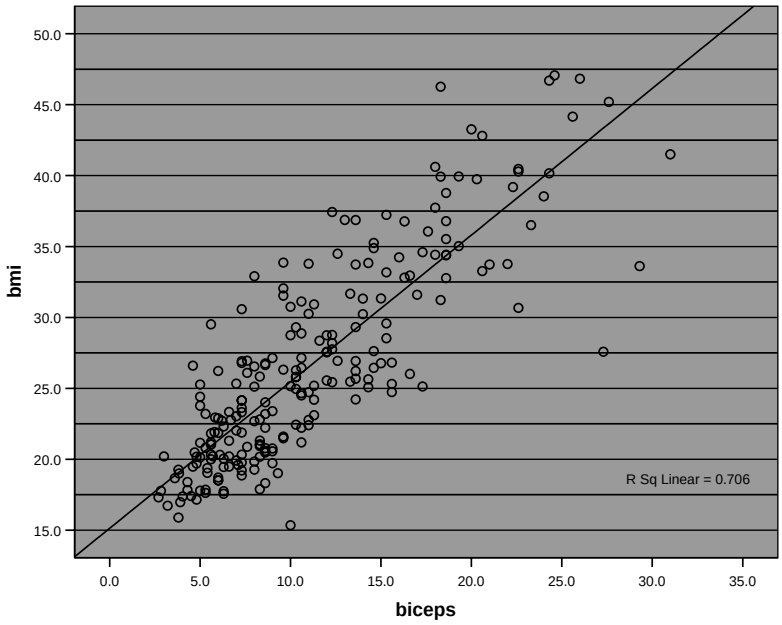


Fig.9: Correlation between BMI and suprailic skinfold thickness in non obese and obese females.

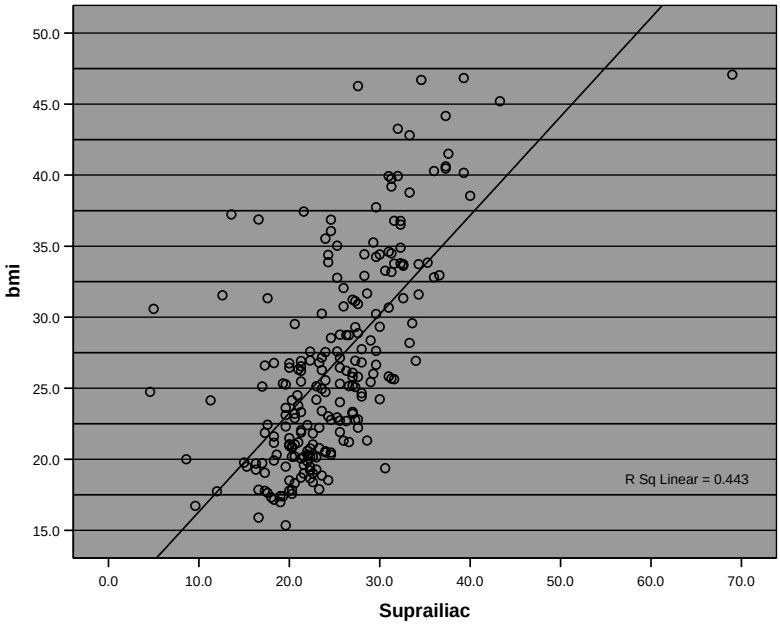


Fig.10: Correlation between BMI and triceps skinfold thickness in non obese and obese females.

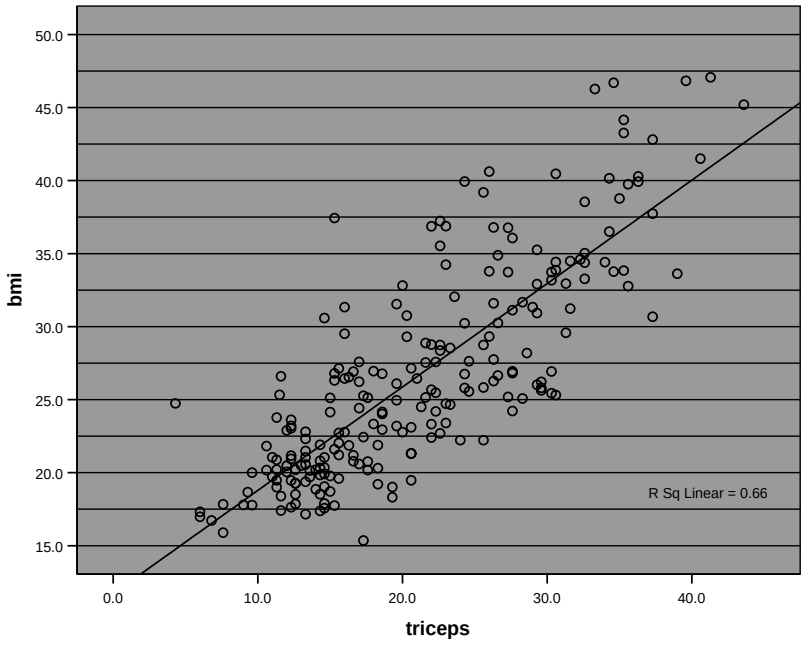


Fig. 11: Waist, thigh and hip circumference of non obese and obese females.

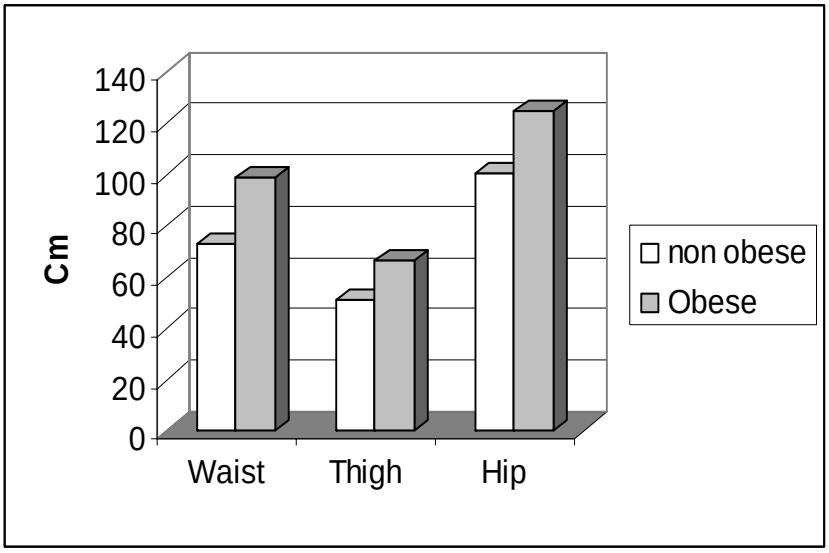


Fig. 12: WHR in all participants of the study, cut-off point >0.85.

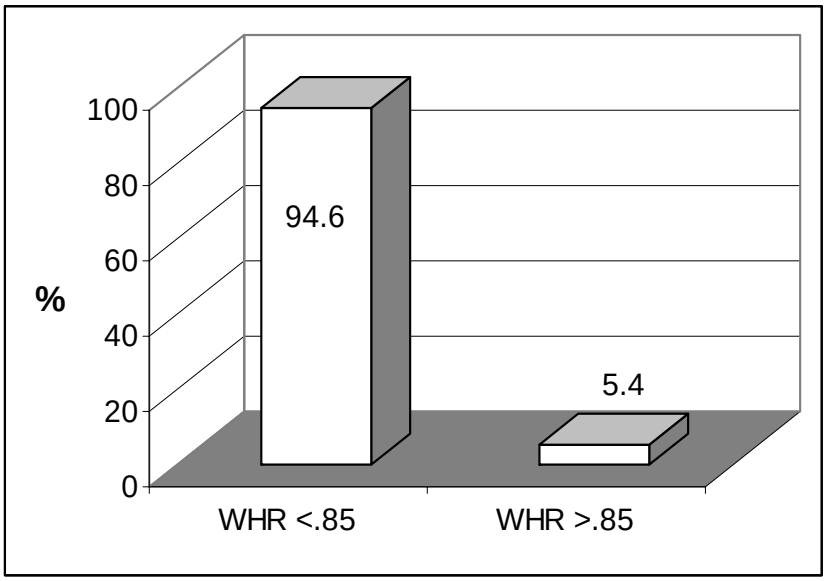


Fig. 13: WHR in obese subjects cut-off point >0.85.

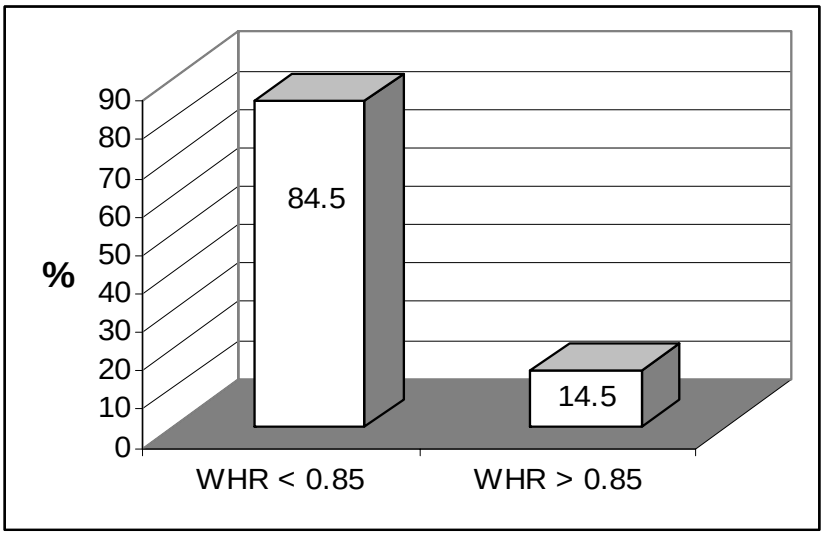


Fig. 14: The percentage of high and normal blood pressure in obese during systole and diastole.

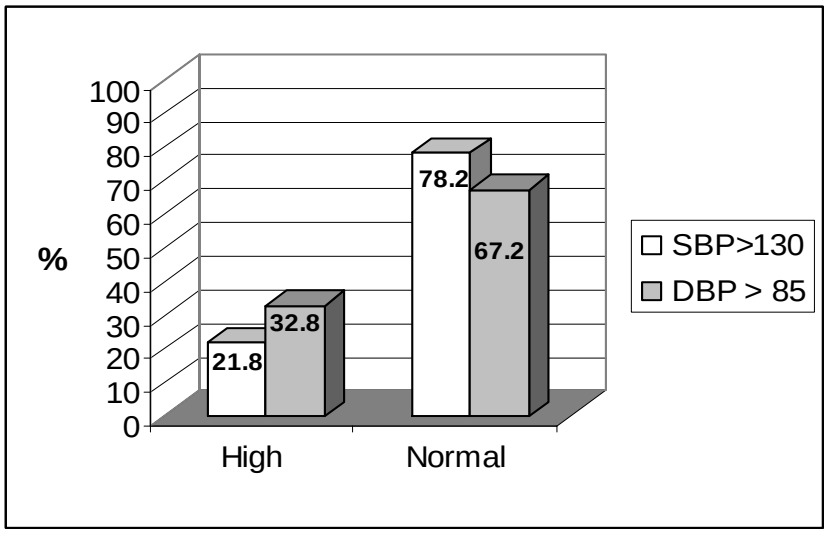


Fig. 15: Correlation between waist circumference and fat mass in non obese and obese females.

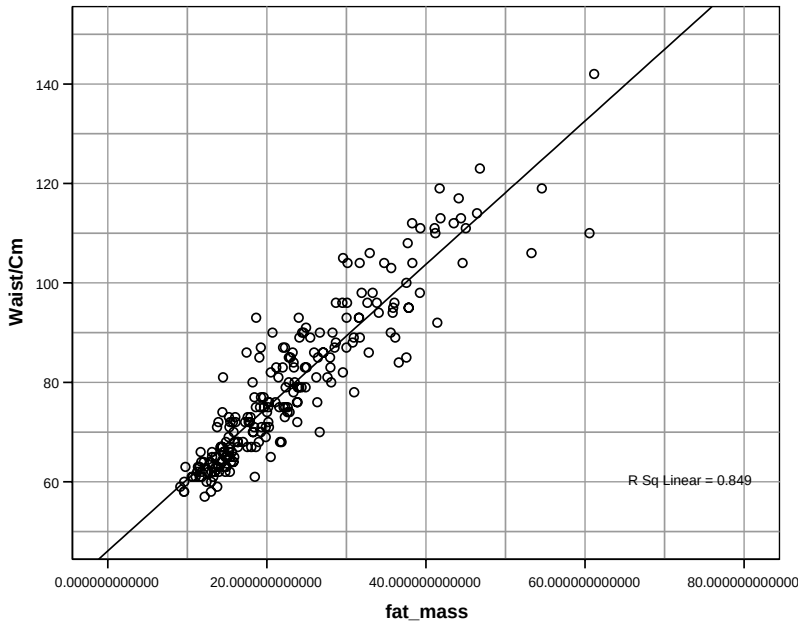


Fig. 16: C- reactive protein level among obese females (CRP <3 mg/l normal & CRP ≥ 3mg/l high)

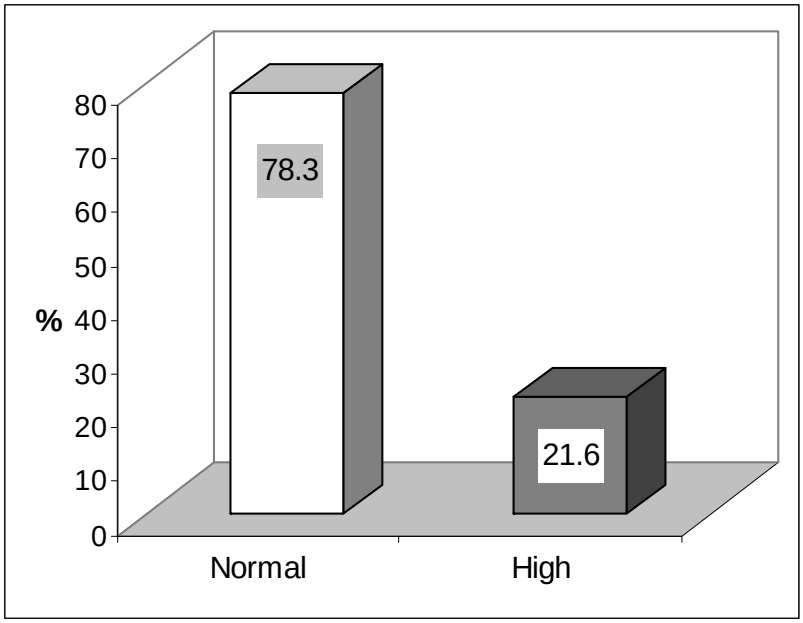
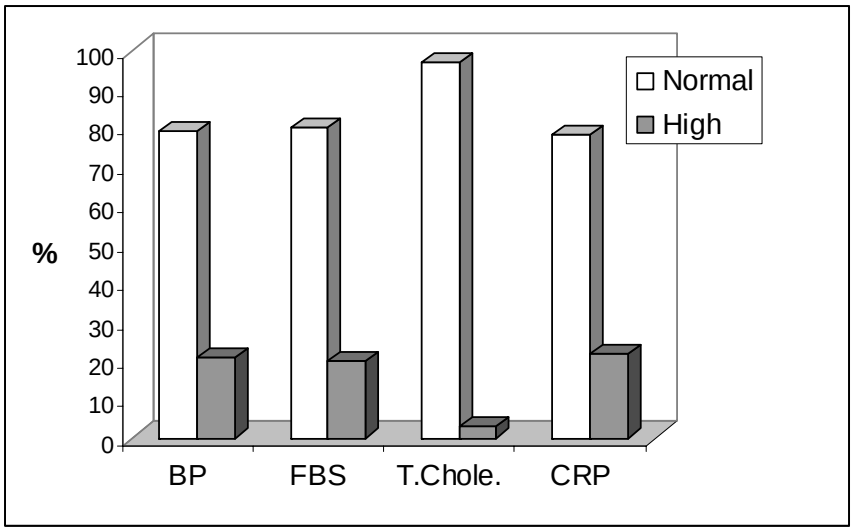


Fig. 17: Blood pressure, fasting blood sugar, total cholesterol and CRP among obese females.(CRP <3 mg/l normal & CRP ≥ 3mg/l high)



CHAPTER FOUR

Discussion

Obesity has become a leading global public health problem. However, very little is known about its incidence, causes and health effect in Africa. Policy makers, donors, and researchers hesitated to attend to obesity in Africa due to the huge burden of infectious diseases associated with the high prevalence of hunger and malnutrition and acute infections in the continent. Recent studies, however, has shown that obesity has increased among both the poor and rich developing countries [209].

The aims in this study to examine the associations of several adiposity measures including total adiposity (BMI and %Fat), body fat distribution (waist, hip, wrist & thigh circumference), and total fat mass with lipid profile parameters and inflammatory markers (CRP and IL-6) in young females, in an attempt to make use of this data as predictor of cardiovascular diseases.

All participants of this study were young non pregnant and non lactating medical students (table 5).

Anthropometry is one of the basic tools for assessing nutritional status [210]. In this study we applied the anthropometric measurements which are most commonly used in public health surveys and clinical screening. These measures were found to be practical, simple, available, and importantly accurate. The methods included in the study are skin fold, waist, hip, thigh circumference measurement, various height and weight based indices or weight for height body mass index. Increased degree of adiposity or obesity is correlated with increased anthropometric measurements [211]. In this study anthropometric measurements were significantly increased in obese compared to non obese females with exception of height (table 5). These findings reflected the prominent contribution of increased body fat to the increase in most anthropometric measurement. These findings were in line with Gibson (1990) and William and Anderson (1993) [212] who reported that increased body weight is associated with increases of the different anthropometric parameters.

The relation of $\log_{10}$ weight for height in the non obese subjects is similar to one calculated by the formula [$\log_{10}$ weight (kg) - 0.8x height (m) + 0.4], this formula have been shown to describe these relation among African and European populations [213]. The $\log_{10}$ weight for obese females was significantly higher than $\log_{10}$ weight for height predicted by the above mentioned formula (Table 6). However, there is no significant difference in the height between this study groups.

The average height of the participant in the study is similar to that reported by Sukkar *et al* [214], who studiedFur tribe women and in line with values of a study curried in females in Gazira University [215]. The average height of the study participants is similar to that of Cameroon, and South African females, on the other hand it is less than that of Spanish, and German females of the same age group [216-219].

Significant difference in weight was reported between non obese and obese subjects in this study (Table 5). The body weight of an individual is however influenced by his stature, hence there is a need for nutritional status index of acceptable body weight for height. Values of body weight adjusted for height, referred to as body mass index. The BMI was calculated from weight in (kg) divided by height in meter square [$W_{(kg)}/H_{(m^2)}$]. BMI for obese females was found to be significantly higher than that of non obese females. (Table 5)

The WHO (1998) defines obesity as a condition with excess body fat to the extent that health and well being are adversely affected. However, many recent reports have demonstrated the limitation of BMI for estimating body fat [220]. The results obtained in this study were in agreement with the previous reports. According to the universal cut-off BMI points, 39% of the participants in this study were obese. The percentage of obese increased to 51.5%, when the participants were classified according to their %BF obtained by skinfold thickness using cut-off point of >30 for obese (Fig. 4). The BMI was found to be positively correlated to % BF (Fig. 5). These findings suggest that a high body fat exist within the sample, despite an acceptable BMI. The results documented the

previous study carried in females medical students in University of Khartoum, in which the percentage of obesity was found to be higher on classifying females by %BF compared to BMI [221]. Similar results have also been reported by Wang *et al* (1994) for Asians [222], and by Deurenbeng *et al* (2000) for Chinese Malays and Indians in Singapore, [223] but not for Caucasian [224].

For further understanding of the participants female's body composition, the body fat distribution indices were examined. The average WHR of participants was 0.74, and 94.6% of them had a ratio less than 0.85 (Fig. 12). In addition the mean WHR for obese subjects was 0.87, and 84.5% of them had WHR less than 0.85 (Fig. 13), WHR was found to be positively correlated with % fat mass and more strongly with BMI (Table 11). These measures reflect decrease accommodation of fat in the abdominal region. Many studies used the WHR to measure the abdominal fat. However, others considered waist circumference to be a better marker of abdominal fat content than WHR [225, 226]. Only 26% of this study participants, had waist circumference of more than 88cm. High WC is associated with high fat mass (Fig. 11) and it was associated with high disease risk [227].

The obtained data show differences in evaluation of obesity between BMI, %BF and WC (Fig. 4), however, WC, TC, and HC were generally strongly correlated with BMI, suggesting that measures of obesity based on these parameters provide comparable results (Table 8, and Fig. 7). Although there was an undesirably high level of body fat in the participants, fortunately it was distributed at the lower part of the body, and may be associated with low obesity related risks.

Blood pressure

Obesity is associated with high risk of metabolic and cardiovascular diseases that include type 2 diabetes, hypertension, and dyslipidemia [228, 229]. The results of this study showed significant difference in blood pressure measured in obese compared to non obese subjects (Table 5), this is in line with a number of reports studying the prevalence of metabolic syndrome and CVD in different populations [230]. It has also been observed that

blood pressure falls within the normal international range (SBP ≤130 and DBP ≤85 mmHg) in most of obese females in this study (Fig. 14). This finding is consistent with many reports of obesity with normal blood pressure [230]. Such uncomplicated obesity has been shown to be unrelated to metabolic syndrome or CVD.

Biochemical parameters

This study showed higher level of TC, LDL-C, TAG and fasting blood glucose in obese females. HDL-C was significantly low in obese compared to non obese females and it was negatively correlated with the BMI (Table 7 and 9). These results were in line with that obtained by Mohammed (2006) who studied lipid profile in Sudanese women in Medani city,[231] and it is in agreement with reports investigating lipid profiles in different ethnic groups [232-234].

In this study, the mean ± SEM of all lipid profile parameters and fasting blood glucose were within the normal physiological ranges. Despite the well- known association of obesity with features of the metabolic syndrome (insulin resistance, dyslipidemia and hypertension), several investigators confirmed the existence of a subgroup of obese individuals with relatively normal insulin sensitivity and favorable metabolic profile [234]. Such groups of obese have been termed metabolically healthy but obese (MHO) and was defined according to the following parameter: no clinically significant abnormalities on physical examination, normal fasting blood glucose (<100mg/dl), normal blood pressure (SBP <130 mmHg and DBP<85mmHg), normal plasma lipids (TC < 200mg/dl, HDL-C>40 mg/dl for men and >50 mg/dl for women, LDL-C<130mg/dl, TAG<150 mg/dl, and TC/HDL-C<4.4) [234]. In fact MHO metabolic profile is similar to those of young lean women [235]. In this study 80% of the obese females had normal blood pressure, normal fasting blood glucose and normal lipid profile, (Fig. 17 & Table 14). This group fulfils these criteria and accordingly they can be classified as metabolically healthy but obese (MHO).

Inflammatory markers

Interleukin-6 was low in all of the study participants and there was no statistical difference between non obese and obese females. The synthesis of CRP by the liver is largely regulated by the cytokine IL-6, [193] since its levels is normal it will not stimulate CRP synthesis.

The mean CRP levels in the obese females was found significantly higher than that of the non obese females (Table 7), and is correlated with %BF, WC, TC, HC, and more strongly with BMI (Table 12 and 13). High CRP levels were recorded in 21.6% of the obese females, while 78.3% of obese females showed normal levels.(Table 14 & Fig.16) The group who had high CRP (>3 mg/l), [236] had also higher blood pressure, higher BMI, and higher WHR compared to the normal CRP obese group, and they had a tendency to develop obesity risks.

Karelis *et al* (2005) reported lower levels of CRP in postmenopausal women with the MHO phenotype, [234] however, the percentage of obese females with low CRP levels (<3mg/l) in this study was in line with Shin *et al* (2006) who observed lower levels of IL-6, CRP and oxidized LDL-C in MHO individuals [237].

The risks associated with excess fat are thought in part to be more affected by site of fat distribution than the total amount of fat [238]. Specifically, increased abdominal adiposity is accepted as a major risk factor for diabetes mellitus, dyslipidemia, hypertension vascular disease and mortality [239].

The study investigated the contribution of adipose tissue distribution to the variation of plasma lipids and inflammatory markers. The results of this study show that only 14.5% of the obese females have WHR above the cut-off point for women (>0.85) (Fig. 13). All of the obese females had high hip circumference (>107Cm).Thigh circumference was found to be higher in obese females compared to non obese females. (Table 5) This finding indicates that fats in obese females accumulated mainly in the lower part of the body rather than the abdominal region. Larger thigh and hip circumferences reflect

increased femoral and gluteal subcutaneous fat. Particularly in women; these depots have relatively high lipoprotein lipase activity and relatively low rate of basal and stimulated lipolysis [239]. These depots may protect the liver and muscle from high exposure to free fatty acids, through uptake and storage. Seidell *et al* (2001) [240] reported that hip circumference was independently and negatively associated with several cardiovascular risk factors, including fasting insulin and glucose levels.

Excessive fatty acid release by adipose tissue has been linked to insulin resistance and dyslipidemia. However, the gluteal fat depot contribute less than other fat depots to fatty acids levels [241]. This might explain the association between the higher risks with abdominal body fat compared with lower body fat. On the other hand the MHO phenotype has been reported in the scientific literature and defined as subgroups of obese individual with relatively normal insulin sensitivity, normal blood pressure and normal lipid profile. Most of the obese females in this study were fulfilling the criteria of these subgroups.

Conclusions

All anthropometric measurements except height, showed higher records among obese females compared to non obese females. Obesity in females studied here was associated with an elevated lipid profile and CRP compared to non obese females. IL-6 shows no difference between the two study groups of non obese and obese females.

The study findings indicated that fats in the obese females accumulated mainly in the lower part of the body rather than the abdominal region. Larger thigh and hip circumference could reflect increased femoral and gluteal subcutaneous fat; these depots have relatively high lipoprotein lipase activity and relatively low rate of basal and stimulated lipolysis. In addition, the majority of the studied obese females had normal blood pressure normal fasting blood glucose and normal lipid profile; accordingly they are metabolically healthy but obese (MHO).

Recommendations

1. This study confirms the limitation of BMI for estimating body fat. Therefore, different ethnic groups cut off points should be established.

2. More detailed measures of obesity are needed in prospective studies that relate changes in body composition to changes in metabolic profile.

3. Large hip circumference is associated with a favorable metabolic profile in several ethnic groups. Further research may be required to gain better insight into the underlying mechanisms explaining this association.

4. WHR should be included in the routine clinical assessment of obesity to identify those at increased health risk of metabolic syndrome caused by increased abdominal fat.

References

1. Rimm EB, Stampfer MJ, Giovannucci E, Ascherio A, Spiegelman D, Colditz GA *et al*. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. Am J Epidemiol. 1995 Jun 15;141(12):1117-27.

2. Flatt JP. Dietary fat, carbohydrate balance, and weight maintenance: effects of exercise. Am J Clin Nutr 1987;45(1 Suppl):296-306.

3. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372(6505):425-32.

4. Schwartz MW, Seeley RJ. Seminars in medicine of the Beth Israel Deaconess Medical Center. Neuroendocrine responses to starvation and weight loss. N Engl J Med 1997;336(25):1802-11.

5. Campfield LA, Smith FJ, Burn P. Strategies and potential molecular targets for obesity treatment. Science 1998;280(5368):1383-7.

6. Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. Science 1998;280(5368):1378-83.

7. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. J Clin Invest 1997;100(2):270-8.

8. Billington CJ, Briggs JE, Grace M, Levine AS. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am J Physiol 1991;260(2 Pt 2):R321-7.

9. Prentice AM, Jebb SA. Obesity in Britain: gluttony or sloth? BMJ 1995;311(7002):437-9.

10. Flatt JP. The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. Ann NY Acad Sci 1987;499:104-23.

11. Flatt JP. Importance of nutrient balance in body weight regulation. Diabetes Metab Rev 1988;4(6):571-81.

12. Hellerstein MK, Wu K, Kaempfer S, Kletke C, Shackleton CH. Sampling the lipogenic hepatic acetyl-CoA pool in vivo in the rat. Comparison of xenobiotic probe to values predicted from isotopomeric distribution in circulating lipids and measurement of lipogenesis and acetyl-CoA dilution. J Biol Chem 1991;266(17):10912-9.

13. Schwarz JM, Neese RA, Turner S, Dare D, Hellerstein MK. Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. J Clin Invest 1995;96(6):2735-43.

14. Aarsland A, Chinkes D, Wolfe RR. Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. Am J Clin Nutr 1997;65(6):1774-82.

15. Jequier E, Tappy L. Regulation of body weight in humans. Physiol Rev 1999;79(2):451-80.

16. Lissner L, Heitmann BL. Dietary fat and obesity: evidence from epidemiology. Eur J Clin Nutr 1995;49(2):79-90.

17. Seidell JC, Muller DC, Sorkin JD, Andres R. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. Int J Obes Relat Metab Disord 1992;16(9):667-74.

18. Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF *et al*. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol 1990;259(5 Pt 1):E650-7.

19. Golay A, Felber JP, Meyer HU, Curchod B, Maeder E, Jequier E. Study on lipid metabolism in obesity diabetes. Metabolism 1984;33(2):111-6.

20. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1(7285):785-9.

21. Bjorntorp P. Metabolic implications of body fat distribution. Diabetes care 1991;14(12):1132-43.

22. Jequier E, Schutz Y. Energy expenditure in obesity and diabetes. Diabetes Metab Rev 1988;4(6):583-93.

23. Prentice AM, Black AE, Coward WA, Davies HL, Goldberg GR, Murgatroyd PR *et al*. High levels of energy expenditure in obese women. Br Med J (Clin Res Ed) 1986;292(6526):983-7.

24. Ravussin E, Burnand B, Schutz Y, Jequier E. Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. Am J Clin Nutr 1982;35(3):566-73.

25. Schutz Y, Tremblay A, Weinsier RL, Nelson KM. Role of fat oxidation in the long-term stabilization of body weight in obese women. Am J Clin Nutr 1992;55(3):670-4.

26. Flier JS. Leptin expression and action: new experimental paradigms. Proc Natl Acad Sci U S A. 1997;94(9):4242-5.

27. Ravussin E, Schutz Y, Acheson KJ, Dusmet M, Bourquin L, Jequier E. Short-term, mixed-diet overfeeding in man: no evidence for "luxuskonsumption". Am J Physiol 1985;249(5 Pt 1):E470-7.

28. Mei N. Intestinal chemosensitivity. Physiol Rev 1985;65(2):211-37.

29. Blundell JE. Regulation of energy intake: appetite control and the potential for weight gain. In: Anderson AH, Bouchard C, Lau D, Leiter L, and Mendelson R, editors. Progress in Obesity Research. London: Libbey; 1996. vol 7 p. 215-22.

30. Blundell JE, Lawton CL, Cotton JR, Macdiarmid JI. Control of human appetite: implications for the intake of dietary fat. Annu Rev Nutr 1996;16:285-319.

31. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G *et al*. The response to long-term overfeeding in identical twins. N Engl J Med 1990;322(21):1477-82.

32. De Castro JM. Socio-cultural determinants of meal size and frequency. Br J Nutr. 1997;77 Suppl 1:S39-54; discussion S54-5.

33. Bray GA. 1989 McCollum Award lecture. Genetic and hypothalamic mechanisms for obesity-finding the needle in the haystack. Am J Clin Nutr 1989;50(5):891-902.

34. Bray GA, Inoue S, Nishizawa Y. Hypothalamic obesity. The autonomic hypothesis and the lateral hypothalamus. Diabetologia 1981;20 Suppl:366-77.

35. Brown FD, Fessler RG, Rachlin JR, Mullan S. Changes in food intake with electrical stimulation of the ventromedial hypothalamus in dogs. J Neurosurg 1984;60(6):1253-7.

36. Assimacopoulos-Jeannet F, Jeanrenaud B. The hormonal and metabolic basis of experimental obesity. Clin Endocrinol Metab 1976;5(2):337-65.

37. Leibowitz S F. Central physiological determinants of eating behavior and weight. In: Brownell KD, and Fairburn CG, editors. Eating Disorders and Obesity. London: Guilford; 1995. p. 3-7.

38. Leibowitz SF. Hypothalamic neuropeptide Y, galanin, and amines. Concepts of coexistence in relation to feeding behavior. Ann NY Acad Sci 1989;575:221-33; discussion 233-25.

39. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K *et al*. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996;379(6560):69-72.

40. Langley SC, Rickett GW, Hunt A, Kelly FJ, Postle AD, York DA. Effects of the glucocorticoid agonist, RU28362, and the antagonist RU486 on lung phosphatidylcholine and antioxidant enzyme development in the genetically obese Zucker rat. Biochem Pharmacol 1993;45(3):543-51.

41. Friedman MI. Body fat and the metabolic control of food intake. Int J Obes 1990;14 Suppl 3:53-66; discussion 66-57.

42. Langhans W, Scharrer E. Evidence for a vagally mediated satiety signal derived from hepatic fatty acid oxidation. J Auton Nerv Syst 1987;18(1):13-8.

43. Knoll J. Satietin: a highly potent anorexogenic substance in human serum. Physiol Behav 1979;23(3):497-502.

44. Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR *et al*. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science 1987;237(4813):402-5.

45. Sanchis D, Balada F, del Mar Grasa M, Virgili J, Peinado J, Monserrat C *et al*. Oleoyl-estrone induces the loss of body fat in rats. Int J Obes Relat Metab Disord 1996;20(6):588-94.

46. Black PR, Brooks DC, Bessey PQ, Wolfe RR, Wilmore DW. Mechanisms of insulin resistance following injury. Ann Surg 1982;196(4):420-35.

47. Blundell JE, Cotton JR, Delargy H, Green S, Greenough A, King NA *et al*. The fat paradox: fat-induced satiety signals versus high fat overconsumption. Int J Obes Relat Metab Disord 1995;19(11):832-5.

48. Welch I, Saunders K, Read NW. Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers. Gastroenterology 1985;89(6):1293-7.

49. Smith GP, Jerome C, Norgren R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am J Physiol 1985;249(5 Pt 2):R638-41.

50. Erlanson-Albertsson C, Mei J, Okada S, York D, Bray GA. Pancreatic procolipase propeptide, enterostatin, specifically inhibits fat intake. Physiol Behav 1991;49(6):1191-4.

51. Mo-suwan L, Junjana C, Puetpaiboon A. Increasing obesity in school children in a transitional society and the effect of the weight control program. Southeast Asian J Trop Med Public Health 1993;24(3):590-4.

52. Popkin BM, Paeratakul S, Zhai F, Ge K. A review of dietary and environmental correlates of obesity with emphasis on developing countries. Obes Res 1995;3 Suppl 2:145s-153s.

53. Dreon DM, Frey-Hewitt B, Ellsworth N, Williams PT, Terry RB, Wood PD. Dietary fat:carbohydrate ratio and obesity in middle-aged men. Am J Clin Nutr 1988;47(6):995-1000.

54. Tremblay A, Plourde G, Despres JP, Bouchard C. Impact of dietary fat content and fat oxidation on energy intake in humans. Am J Clin Nutr 1989;49(5):799-805.

55. Gibney MJ. Epidemiology of obesity in relation to nutrient intake. Int J Obes Relat Metab Disord 1995;19 Suppl 5:S1-3.

56. Nelson LH, Tucker LA. Diet composition related to body fat in a multivariate study of 203 men. J Am Diet Assoc 1996;96(8):771-7.

57. Prewitt TE, Schmeisser D, Bowen PE, Aye P, Dolecek TA, Langenberg P *et al*. Changes in body weight, body composition, and energy intake in women fed high- and low-fat diets. Am J Clin Nutr 1991;54(2):304-310.

58. Reducing risks promoting healthy life. In: Barbara C, Angela H, editors. World health report. World Health Organization; 2002. p. 60-1

59. The foundation for professional development. Manual for certificate in Management of obesity. South Africa Medical Association; September 2001.

60. International Obesity Task Force data, based on population weighted estimates from published and unpublished surveys. 1990-2002 (latest available using IOTF-recommended cut-offs for overweight and obesity) [Online]. 2004 [cited 2004 Jan 28]; Available from: URL: http://www.iotf.org/.

61. National Institutes of Health, National Heart, Lung, and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—the evidence report. National Institutes of Health, National Heart, Lung, and Blood Institute. Obes Res 1998; 6(Suppl 2):53–4.

62. Walker ARP. Epidemiology and health implications of obesity in Southern Africa. In: Steyn IF, Parow S, editors. Chronic diseases of lifestyle in South Africa: review of research and identification of essential health resource priority. Cape Town: Medical Research, The National Institute of Health; 1995. p. 73-85.

63. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. Am J Clin Nutr 2004;79(3):379-84.

64. Dalton M, Cameron AJ, Zimmet PZ, Shaw JE, Jolley D, Dunstan DW *et al*. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. J Intern Med 2003;254(6):555-63.

65. Guidelines for the prevention and management of overweight and obesity in South Africa. South Africa: Southern African Society for the Study of Obesity (SASSO); 2006.

66. Epstein LH, Wing RR, Koeske R, Valoski A. Long-term effects of family-based treatment of childhood obesity. J Consult Clin Psychol 1987;55(1):91-5.

67. Brown DD. International Obesity Task Force Report [Online]. 2003 [cited 2004 Jan 28] Available from: URL: http://www.iotf.org/.

68. American Obesity Association. The extent of the problem. Fact sheets on obesity and overweight[Online]. 2002 [28 th Jan. 2004] Available from: URL: http://www.mchlibrary.info/KnowledgePaths/kp_overweight.html

69. Martorell R, Khan LK, Hughes ML, Grummer-Strawn LM. Obesity in Latin American women and children. J Nutr 1998;128(9):1464-73.

70. Gill TP. Key issues in the prevention of obesity. Br Med Bull 1997;53(2):359-88.

71. Filozof C, Gonzalez C. Predictors of weight gain: the biological-behavioural debate. Obes Rev 2000;1(1):21-6.

72. Egger G, Swinburn B. An "ecological" approach to the obesity pandemic. BMJ (Clinical research ed) 1997;315(7106):477-80.

73. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. Br Med Bull 1997;53(2):238-52.

74. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000, 894:i-xii, 1-253.

75. Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord 1998;22(1):39-47.

76. Stam-Moraga MC, Kolanowski J, Dramaix M, De Henauw S, De Bacquer D, De Backer G *et al*. Trends in the prevalence of obesity among Belgian men at work, 1977-1992. Int J Obes Relat Metab Disord 1998;22(10):988-92.

77. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol 2000;89(1):81-8.

78. Rolland-Cachera MF, Cole TJ, Sempe M, Tichet J, Rossignol C, Charraud A. Body Mass Index variations: centiles from birth to 87 years. Eur J Clin Nutr 1991;45(1):13-21.

79. Boyle CA, Dobson AJ, Egger G, Magnus P. Can the increasing weight of Australians be explained by the decreasing prevalence of cigarette smoking? Int J Obes Relat Metab Disord 1994;18:55-60.

80. Seidell JC, Verschuren WM, Kromhout D. Prevalence and trends of obesity in The Netherlands 1987-1991. Int J Obes Relat Metab Disord 1995;19(12):924-7.

81. Stam-Moraga MC, Kolanowski J, Dramaix M, De Backer G, Kornitzer MD. Sociodemographic and nutritional determinants of obesity in Belgium. Int J Obes Relat Metab Disord 1999;23 Suppl 1:1-9.

82. Rissanen A, Heliovaara M, Aromaa A. Overweight and anthropometric changes in adulthood: a prospective study of 17,000 Finns. Int J Obes 1988;12(5):391-401.

83. Williamson DF, Kahn HS, Remington PL, Anda RF. The 10-year incidence of overweight and major weight gain in US adults. Arch Intern Med 1990;150(3):665-72.

84. Lewis CE, Smith DE, Wallace DD, Williams OD, Bild DE, Jacobs DR Jr. Seven-year trends in body weight and associations with lifestyle and behavioral characteristics in black and white young adults: the CARDIA study. Am J Public Health 1997;87(4):635-42.

85. Guo SS, Zeller C, Chumlea WC, Siervogel RM. Aging, body composition, and lifestyle: the Fels Longitudinal Study. Am J Clin Nutr 1999;70(3):405-11.

86. Heitmann BL, Garby L. Patterns of long-term weight changes in overweight developing Danish men and women aged between 30 and 60 years. Int J Obes Relat Metab Disord 1999;23(10):1074-8.

87. Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? Am J Epidemiol 1996;143(3):228-39.

88. Deurenberg P, Yap M, van Staveren WA. Body mass index and percent body fat: a meta analysis among different ethnic groups. Int J Obes Relat Metab Disord 1998;22(12):1164-71.

89. Lissner L. Psychosocial aspects of obesity: Individual and societal perspectives. Scand J Nutr 1997;41:75-9.

90. Sobal J, Stunkard AJ. Socioeconomic status and obesity: a review of the literature. Psychol Bull 1989;105(2):260-75.

91. Wardle J, Griffith J. Socioeconomic status and weight control practices in British adults. J Epidemiol Community Health 2001;55(3):185-90.

92. Molarius A, Seidell JC, Sans S, Tuomilehto J, Kuulasmaa K. Educational level, relative body weight, and changes in their association over 10 years: an international perspective from the WHO MONICA Project. Am J Public Health 2000;90(8):1260-8.

93. Sundquist J, Johansson SE. The influence of socioeconomic status, ethnicity and lifestyle on body mass index in a longitudinal study. Int J Epidemiol 1998;27(1):57-63.

94. Kahn HS, Williamson DF. The contributions of income, education and changing marital status to weight change among US men. Int J Obes 1990;14(12):1057-68.

95. Bjorkelund C, Lissner L, Andersson S, Lapidus L, Bengtsson C. Reproductive history in relation to relative weight and fat distribution. Int J Obes Relat Metab Disord 1996;20(3):213-9.

96. Wamala SP, Wolk A, Orth-Gomer K. Determinants of obesity in relation to socioeconomic status among middle-aged Swedish women. Prev Med 1997;26(5 Pt 1):734-44.

97. Lahmann PH, Lissner L, Gullberg B, Berglund G. Sociodemographic factors associated with long-term weight gain, current body fatness and central adiposity in Swedish women. Int J Obes Relat Metab Disord 2000;24(6):685-94.

98. Smith BK, York DA, Bray GA. Chronic cerebroventricular galanin does not induce sustained hyperphagia or obesity. Peptides 1994;15(7):1267-72.

99. Kahn HS, Williamson DF. Abdominal obesity and mortality risk among men in nineteenth-century North America. Int J Obes Relat Metab Disord 1994;18(10):686-91.

100. Ohlin A, Rossner S. Trends in eating patterns, physical activity and socio-demographic factors in relation to postpartum body weight development. Br J Nutr 1994;71(4):457-70.

101. Rossner S, Ohlin A. Pregnancy as a risk factor for obesity: lessons from the Stockholm Pregnancy and Weight Development Study. Obes Res 1995;3 Suppl 2:267s-275s.

102. Bray GA, Popkin BM. Dietary fat intake does affect obesity! Am J Clin Nutr 1998;68(6):1157-73.

103. Hill JO, Melanson EL, Wyatt HT. Dietary fat intake and regulation of energy balance: implications for obesity. J Nutr 2000;130(2S Suppl):284S-288S.

104. Poppitt SD. Energy density of diets and obesity. Int J Obes Relat Metab Disord 1995;19 Suppl 5:S20-6.

105. Rolls BJ. The role of energy density in the overconsumption of fat. J Nutr 2000;130(2S Suppl):268S-271S.

106. Seidell JC. Dietary fat and obesity: an epidemiologic perspective. Am J Clin Nutr 1998;67(3 Suppl):546S-550S.

107. Doucet E, Almeras N, White MD, Despres JP, Bouchard C, Tremblay A. Dietary fat composition and human adiposity. Eur J Clin Nutr 1998;52(1):2-6.

108. Hjartaker A, Lund E. Relationship between dietary habits, age, lifestyle, and socio-economic status among adult Norwegian women. The Norwegian Women and Cancer Study. Eur J Clin Nutr 1998;52(8):565-72.

109. Martinez-Gonzalez MA, Martinez JA, Hu FB, Gibney MJ, Kearney J. Physical inactivity, sedentary lifestyle and obesity in the European Union. Int J Obes Relat Metab Disord 1999;23(11):1192-201.

110. Cooper AR, Page A, Fox KR, Misson J. Physical activity patterns in normal, overweight and obese individuals using minute-by-minute accelerometry. Eur J Clin Nutr 2000;54(12):887-94.

111. Tremblay A, Buemann B, Theriault G, Bouchard C. Body fatness in active individuals reporting low lipid and alcohol intake. Eur J Clin Nutr 1995;49(11):824-31.

112. Fentem PH, Mockett SJ. Physical activity and body composition: what do the national surveys reveal? Int J Obes Relat Metab Disord 1998;22 Suppl 2:S8-14.

113. Salmon J, Bauman A, Crawford D, Timperio A, Owen N. The association between television viewing and overweight among Australian adults participating in varying levels of leisure-time physical activity. Int J Obes Relat Metab Disord 2000;24(5):600-06.

114. Jeffery RW, French SA. Epidemic obesity in the United States: are fast foods and television viewing contributing? Am J Public Health 1998;88(2):277-80.

115. Heaton KW. The epidemiology of gallstones and suggested aetiology. Clin Gastroenterol. 1973 Jan;2(1):67-83.

116. Higgins M, Kannel W, Garrison R, Pinsky J, Stokes J, Hazards of obesity--the Framingham experience. Acta Med Scand 1988;723:23-36.

117. Ducimetiere P, Richard JL. The relationship between subsets of anthropometric upper versus lower body measurements and coronary heart disease risk in middle-aged men. The Paris Prospective Study. I. Int J Obes 1989;13(1):111-21.

118. Kissebah AH, Peiris A, Evans DF. Mechanisms associating body fat distribution with the abnormal metabolic profiles in obesity. In: Berry SM, Blondheim SM, Eliahou HE, Shafrir E, edioters. Recent Advances in Obesity Research. London: Libbey; 1986. p. 54.

119. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J (Clin Res Ed) 1984;289(6454):1257-61.

120. Welin L, Svardsudd K, Wilhelmsen L, Larsson B, Tibblin G. Analysis of risk factors for stroke in a cohort of men born in 1913. N Engl J Med 1987;317(9):521-6.

121. Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR *et al*. A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med 1990;322(13):882-9.

122. Glueck CJ, Taylor HL, Jacobs D, Morrison JA, Beaglehole R, Williams OD. Plasma high-density lipoprotein cholesterol: association with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(4 Pt 2):IV-62-9.

123. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62(5):707-14.

124. Montoye HJ, Epstein FH, Kjelsberg MO. Relationship between serum cholesterol and body fatness. An epidemiologic study. Am J Clin Nutr 1966;18(6):397-406.

125. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. Am J Cardiol 1992;70(7):733-7.

126. Kannel WB, Gordon T, Castelli WP. Obesity, lipids, and glucose intolerance. The Framingham Study. Am J Clin Nutr 1979;32(6):1238-45.

127. Van Itallie TB. Health implications of overweight and obesity in the United States. Ann Intern Med 1985;103(6 (Pt 2)):983-8.

128. Farinaro E, Cortese C, Rubba P, DiMarino L, Mancini M. Overweight and plasma lipoprotein abnormalities in a random sample of the neapolitan population. In: Mancini M LB, Contaldo F, editors. Medical Complications of Obesity. London: Academic Press; 1979. p. 143-50.

129. Stamler J. Overweight, hypertension, hypercholesterolemia and coronary heart disease. In: Mancini M, Lewis B, Contaldo F, editors. Medical Complications of Obesity.. London: Academic Press; 1979. p. 191-216.

130. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 1990;10(4):493-6.

131. Eckel RH, Yost TJ. Weight reduction increases adipose tissue lipoprotein lipase responsiveness in obese women. J Clin Invest 1987;80(4):992-7.

132. Stamler R, Stamler J, Riedlinger WF, Algera G, Roberts RH. Weight and blood pressure. Findings in hypertension screening of 1 million Americans. JAMA 1978;240(15):1607-10.

133. Burton BT, Foster WR, Hirsch J, Van Itallie TB. Health implications of obesity: an NIH Consensus Development Conference. Int J Obes 1985;9(3):155-70.

134. Havlik RJ, Hubert HB, Fabsitz RR, Feinleib M. Weight and hypertension. Ann Intern Med 1983;98(5 Pt 2):855-9.

135. MacMahon SW, Blacket RB, Macdonald GJ, Hall W. Obesity, alcohol consumption and blood pressure in Australian men and women. The National Heart Foundation of Australia Risk Factor Prevalence Study. J Hypertens 1984;2(1):85-91.

136. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest 1975;55(4):845-55.

137. Dornfeld LP, Maxwell MH, Waks A, Tuck M. Mechanisms of hypertension in obesity. Kidney Int 1987;22:S254-8.

138. Knowler WC, Pettitt DJ, Savage PJ, Bennett PH. Diabetes incidence in Pima indians: contributions of obesity and parental diabetes. Am J Epidemiol 1981;113(2):144-56.

139. Zimmet P, Faaiuso S, Ainuu J, Whitehouse S, Milne B, DeBoer W. The prevalence of diabetes in the rural and urban Polynesian population of Western Samoa. Diabetes 1981;30(1):45-51.

140. United States. National Commission on Diabetes. Report of the National Commission on Diabetes to the Congress of the United States. Bethesda, Maryland: U.S. Department of Health, Education and Welfare; 1975. vol. 1 p. 1021-76.

141. Colditz GA, Willett WC, Stampfer MJ, Manson JE, Hennekens CH, Arky RA *et al*. Weight as a risk factor for clinical diabetes in women. Am J Epidemiol 1990;132(3):501-13.

142. Westlund K, Nicolaysen R. Ten-year mortality and morbidity related to serum cholesterol. A follow-up of 3.751 men aged 40-49. Scand J Clin Lab Invest 1972;127:1-24.

143. Rimm AA, Werner LH, Yserloo BV, Bernstein RA. Relationship of obesity and disease in 73,532 weight-conscious women. Public Health Rep 1975;90(1):44-51.

144. Stampfer MJ, Maclure KM, Colditz GA, Manson JE, Willett WC. Risk of symptomatic gallstones in women with severe obesity. Am J Clin Nutr 1992;55(3):652-8.

145. Liddle RA, Goldstein RB, Saxton J. Gallstone formation during weight-reduction dieting. Arch Intern Med 1989;149(8):1750-3.

146. Remmers JE, deGroot WJ, Sauerland EK, Anch AM. Pathogenesis of upper airway occlusion during sleep. J Appl Physiol 1978;44(6):931-8.

147. Sharp JT, Barrocas M, Chokroverty S. The cardiorespiratory effects of obesity. Clin Chest Med 1980;1(1):103-18.

148. Garfinkel L. Overweight and cancer. Ann Intern Med 1985.103(6(Pt 2)):1034-6.

149. de Waard F. Breast cancer incidence and nutritional status with particular reference to body weight and height. Cancer Res 1975;35(11 Pt. 2):3351-6.

150. Lubin F, Ruder AM, Wax Y, Modan B. Overweight and changes in weight throughout adult life in breast cancer etiology. A case-control study. Am J Epidemiol 1985;122(4):579-88.

151. Sidney S, Friedman GD, Siegelaub AB. Thinness and mortality. Am J Public Health 1987;77(3):317-22.

152. Weber M L. Clinical and roentgenological analysis of 150 cases of chronic non specific arthritis. Med. Bull. Vet. Admin 1939; 2:43-60.

153. Sjostrom L, Kvist H, Cederblad A, Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. Am J Physiol 1986, 250(6 Pt 1):E736-45.

154. Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 1987;36(1):54-9.

155. Copstead LC. & Banasik JL. Pathophysiology: Biological and behavioral perspectives. 2nd ed. Philadelphia: W.B. Saunders Company; 2000. p. 950-1.

156. Porth CM. Pathophysiology: concepts of altered health states. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 212-6.

157. Vorona RD, Winn MP, Babineau TW, Eng BP, Feldman HR, Ware JC. Overweight and obese patients in a primary care population report less sleep than patients with a normal body mass index. Arch Intern Med 2005;165(1):25-30.

158. Guyton AC, & Hall JE. Textbook of Medical Physiology. 10th ed. Philadelphia: W.B. Saunders; 2000.

159. William F Ganong. Review of Medical Physiology. 21 ed. Lange Medical Books/ McGraw-Hill 2003..

160. Grundy SM. Nutrition and Coronary Heart Disease. In: Fuster V, Ross R, Topol EJ, editors. Atherosclerosis and coronary artery disease. Philadelphia: Lippincott-Raven; 1996. vol 1. p. 45-68.

161. Santamarina-Fojo S. The familial chylomicronemia syndrome. Endocrinol Metab Clin North Am 1998;27(3):551-67.

162. Choi SY, Fong LG, Kirven MJ, Cooper AD. Use of an anti-low density lipoprotein receptor antibody to quantify the role of the LDL receptor in the

removal of chylomicron remnants in the mouse in vivo. J Clin Invest 1991;88(4):1173-81.

163. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. J Clin Invest 1998;101(3):689-95.

164. Gibbons GF. Assembly and secretion of hepatic very-low-density lipoprotein. Biochem J 1990;268(1):1-13.

165. van Dijk KW, Hofker MH, Havekes LM. Dissection of the complex role of apolipoprotein E in lipoprotein metabolism and atherosclerosis using mouse models. Curr Atheroscler Rep 1999;1(2):101-7.

166. Demant T, Carlson LA, Holmquist L, Karpe F, Nilsson-Ehle P, Packard CJ *et al*. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. J Lipid Res 1988;29(12):1603-11

167. Auwerx JH, Marzetta CA, Hokanson JE, Brunzell JD. Large buoyant LDL-like particles in hepatic lipase deficiency. Arteriosclerosis. 1989 May-Jun;9(3):319-25.

168. Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. Arterioscler Thromb 1993;13(2):147-53.

169. von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol. 2001;21(1):13-27.

170. Asztalos BF. High-density lipoprotein metabolism and progression of atherosclerosis: new insights from the HDL Atherosclerosis Treatment Study. Curr Opin Cardiol 2004;19(4):385-91.

171. Young SG, Fielding CJ. The ABCs of cholesterol efflux. Nat Genet 1999;22(4):316-8.

172. Oram JF, Lawn RM. ABCA1. The gatekeeper for eliminating excess tissue cholesterol. J Lipid Res 2001;42(8):1173-9.

173. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC *et al*. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 1999;22(4):352-5.

174. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M *et al*. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999;22(4):336-45.

175. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W *et al*. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 1999;22(4):347-51.

176. Gotto AM, Jr. High-density lipoprotein cholesterol and triglycerides as therapeutic targets for preventing and treating coronary artery disease. Am Heart J 2002;144(6 Suppl):S33-42.

177. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes care 1993;16(2):434-44.

178. Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, Heinonen OP *et al*. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 1992;85(1):37-45.

179. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med 1993;94(4):350-6.

180. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93(3):189-99.

181. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM *et al*. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96(12):4219-25.

182. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351(9096):88-92.

183. Benjamin IJ, McMillan DR. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res 1998;83(2):117-32.

184. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282(22):2131-5.

185. Jang Y, Lincoff AM, Plow EF, Topol EJ. Cell adhesion molecules in coronary artery disease. J Am Coll Cardiol 1994;24(7):1591-601.

186. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995.95(5):2111-9.

187. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS *et al*. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997;82(12):4196-200.

188. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990;265(3):621-36.

189. Du Clos TW. Function of C-reactive protein. Ann Med 2000;32(4):274-8.

190. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102(18):2165-8.

191. Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol 2001;280(6):E827-47.

192. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol 2000;529 Pt 1:237-42.

193. Bastard JP, Jardel C, Delattre J, Hainque B, Bruckert E, Oberlin F. Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects. Circulation 1999;99(16):2221-2.

194. World Health Organization. MONICA Manual: CVD/MNC. Geneva, Switzerland: World Health Organization 1990.

195. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486-97.

196. Nicklas BJ, Penninx BW, Cesari M, Kritchevsky SB, Newman AB, Kanaya AM *et al*. Association of visceral adipose tissue with incident myocardial infarction in older men and women: the Health, Aging and Body Composition Study. Am J Epidemiol 2004;160(8):741-9.

197. Nicklas BJ, Rogus EM, Colman EG, Goldberg AP. Visceral adiposity, increased adipocyte lipolysis, and metabolic dysfunction in obese postmenopausal women. Am J Physiol 1996;270(1 Pt 1):E72-8.

198. Recommendations concerning body measurements for the characterization of nutritional status. Hum Biol 1956;28(2):111-23.

199. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32(1):77-7.

200. Siri WE. Body composition from fluid spaces density. Analysis of methods in techniques for measuring body consumption. In: Brozek J , editor. Techniques for Measuring Body Composition. Washington DC: National Academy of Science; 1961. p. 223-47.

201. Tietz NW. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders Company; 1995. p. 610-11.

202. Jacobs NJ, Vandemark PJ. The purification and properties of the alpha-glycerophosphate-oxidizing enzyme of Streptococcus faecalis 10C1. Arch Biochem Biophys 1960;88:250-5.

203. Warnick GR, Mayfield C, Benderson J, Chen JS, Albers JJ. HDL cholesterol quantitation by phosphotungstate-Mg2+ and by dextran sulfate-Mn2+-polyethylene glycol precipitation, both with enzymic cholesterol assay compared with the lipid research method. Am J Clin Path 1982;78(5):718-23.

204. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499-502.

205. Krause KJ: C-reactive protein-a screening test for coronary disease? J Insur Med (New York, NY) 2001;33(1):4-11.

206. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH *et al*. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999;106(5):506-12.

207. Bowden CH. The Estimation of Blood Galactose Using a Glucose Oxidase-Catalase Reagent. J Clin Pathol 1963;16:470-2.

208. Diagnosis and classification of diabetes mellitus. Diabetes care 2007;30 Suppl 1:S42-7.

209. Asfaw A. The effects of obesity on doctor-diagnosed chronic diseases in Africa: empirical results from Senegal and South Africa. J Public Health Policy 2006;27(3):250-64.

210. Bachrach-Lindstrom M, Unosson M, Ek AC, Arnqvist HJ. Assessment of nutritional status using biochemical and anthropometric variables in a nutritional intervention study of women with hip fracture. Clin Nutr. 2001 Jun;20(3):217-23.

211. Williams SR, & Anderson SL. Nutrition and Diet Therapy. Torinto: Mosby, Year book Ine; 1993. p. 43-81, 124-6.

212. Gibson RS. Principal of nutritional assessment. Oxford: Oxford University Press; 1990. p. 37-9, 178-200.

213. Davies CT, Mbelwa D, Dore C. Physical growth and development of urban and rural East African children, aged 7-16 years. Ann Hum Biol 1974;1(3):257-68.

214. Sukkar MY, Vokac Z, Nagdi S, Khogali A. The nutritional status of the Fur people of Jabel Marra. Afr J Med Med Sci 1976;5(4):245-254.

215. Aliya El-Amin Mohammed Al-Badawi. A study of obesity in women medical students University of Gezira, Sudan. MSc. thesis. Medani: University of Gazira; 2007.

216. Kamadjeu RM, Edwards R, Atanga JS, Kiawi EC, Unwin N, Mbanya JC. Anthropometry measures and prevalence of obesity in the urban adult population of Cameroon: an update from the Cameroon Burden of Diabetes Baseline Survey. BMC public health 2006;6:228.

217. Palmer IM, Schutte AE, Huisman HW, Van Rooyen JM, Schutte R, Malan L *et al*. A comparison of uric acid levels in Black African vs Caucasian women from South Africa: the POWIRS study. Ethn Dis 2007;17(4):676-81.

218. Quiroga Valle, Gloria. Height evolution in Spain, 1893-1954. An analysis by regions and professions. In: Komlos J, Baten J, editors. The Biological Standard of Living in Comparative Perspectiv. Stuttgart Franz Steiner 1998.

219. Baten, Joerg. Anthropometrics, consumption and leisure: The standard of living. In: Sheilagh Ogilvie, Richard Overy, editors. Germany: A New Social and Economic HistoryI. London: Edward Arnold; 2003. vol 3. p. 383-422.

220. Jones JS. Life in the 21st century - a vision for all. S Afr Med J. 1998 Jun;88(6):674.

221. Ahmed Abdalla El Ramadi. Skinfold thickness versus body mass index in detection of obesity among young adults. MSc. thesis. Khartoum: University of Khartoum; 2005.

222. Wang J, Thornton JC, Burastero S, Shen J, Tanenbaum S, Heymsfield SB *et al*. Comparisons for body mass index and body fat percent among Puerto Ricans, blacks, whites and Asians living in the New York City area. Obes Res 1996;4(4):377-84.

223. Deurenberg-Yap M, Schmidt G, van Staveren WA, Deurenberg P. The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. Int J Obes Relat Metab Disord 2000;24(8):1011-7.

224. Gallagher D, Visser M, De Meersman RE, Sepulveda D, Baumgartner RN, Pierson RN *et al*. Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. J Appl Physiol 1997;83(1):229-39.

225. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A *et al*. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am J Cardiol 1994;73(7):460-8.

226. Chan DC, Watts GF, Barrett PH, Burke V. Waist circumference, waist-to-hip ratio and body mass index as predictors of adipose tissue compartments in men. QJM 2003;96(6):441-7.

227. Farin HM, Abbasi F, Reaven GM. Comparison of body mass index versus waist circumference with the metabolic changes that increase the risk of cardiovascular disease in insulin-resistant individuals. Am J Cardiol 2006;98(8):1053-6.

228. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA 2004;291(23):2847-50.

229. Kain J, Vio F, Albala C. Obesity trends and determinant factors in Latin America. Cad Saude Publica. 2003;19 Suppl 1:S77-86. Epub 2003 Jul 21.

230. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Prevalence of uncomplicated obesity in an Italian obese population. Obes Res 2005;13(6):1116-22.

231. Mohammed N. Anthropometric and biochemical studies in obese Sudanese women 40- 50 years old: A case study-Medani area, Gezira State. PhD. thesis. Medani: Gezira University; 2006.

232. Sims RC, Madhere S, Gordon S, Clark E, Jr., Abayomi KA *et al*. Relationships among blood pressure, triglycerides and verbal learning in African Americans. J Natl Med Assoc 2008;100(10):1193-8.

233. Sundborn G, Metcalf PA, Gentles D, Scragg RK, Schaaf D, Dyall L *et al*. Ethnic differences in cardiovascular disease risk factors and diabetes status for Pacific ethnic groups and Europeans in the Diabetes Heart and Health Survey (DHAH) 2002-2003, Auckland New Zealand. N Z Med J 2008;121(1281):28-39.

234. Karelis AD, Faraj M, Bastard JP, St-Pierre DH, Brochu M, Prud'homme D *et al*. The metabolically healthy but obese individual presents a favorable inflammation profile. J Clin Endocrinol Metab 2005;90(7):4145-50.

235. Ferreira I, Snijder MB, Twisk JW, van Mechelen W, Kemper HC, Seidell JC *et al*. Central fat mass versus peripheral fat and lean mass: opposite (adverse versus favorable) associations with arterial stiffness? The Amsterdam Growth and Health Longitudinal Study. J Clin Endocrinol Metab. 2004;89(6):2632-9.

236. Miller M, Zhan M, Havas S. High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the Third National Health and Nutrition Examination Survey. Arch Intern Med 2005;165(18):2063-8.

237. Shin MJ, Hyun YJ, Kim OY, Kim JY, Jang Y, Lee JH. Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. Int J Obes (Lond). 2006 Oct;30(10):1529-34. Epub 2006 Mar 21

238. Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ *et al*. Abdominal adiposity and coronary heart disease in women. JAMA 1998;280(21):1843-1848.

239. Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK *et al*. Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 1982;54(2):254-60.

240. Seidell JC, Perusse L, Despres JP, Bouchard C. Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. Am J Clin Nutr 2001;74(3):315-321.

241. Tan GD, Goossens GH, Humphreys SM, Vidal H, Karpe F. Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans. Obes Res 2004;12(1):114-8.